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The Effect of Holothurin on Leucocyte Migration

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Crude Holothurin, purified Holothurin A, and desulfated Holothurin A, water soluble steroid saponins from the Cuvierian organs of the Bahamian sea cucumber *Actinopyga agassizi* Selenka, cause a stimulation of leucocyte migration from buffy layer in capillary tubes in relatively low concentrations and an inhibition of the movements at higher concentrations. The inhibitory properties are compared with similar effects by Ouabain, a non-hemolytic saponin, and by liberated hemoglobin.

The Holothurins had no effect on the migration pattern of the leucocytes in the presence of anaerobic and aerobic inhibitors.

The effect of Holothurin on the amoeboid movements of the leucocytes is discussed in relation to surface alterations and cell membrane permeability.

Introduction

THE MAIN PURPOSE of this study was to obtain additional information on the biological properties of Holothurin and its fractions. Many toxic materials obtained from plant and animal tissues cause similar host responses when released from combination with cellular components. The effects of crude Holothurin, Holothurin A, and desulfated Holothurin were studied in systems of mammalian leucocytes present in whole blood or in saline suspensions of white blood cells using the migration technique of Ketchel and Favour (1955). Comparative experiments were made with the saponin Ouabain, while hemolytic properties were investigated in relation to the inhibition of leucocyte activity. By the use of aerobic and anaerobic inhibitors, an estimate was made of biochemical pathways associated with a stimulation of leucocyte migration.

Holothurin is a water soluble, steroid saponin with surface-active properties found in the Cuvierian organ of the Bahamian and West Indian sea cucumber *Actinopyga agassizi* Selenka. The active principle may be obtained from granules in branching filaments of Cuvierian tubules. Crude Holothurin represents dried tubules and analysis shows the presence of glycosides, pigment, cholesterol, insoluble proteins, salts, free amino acids, and polypeptides (Nigrelli and Jakowska, 1960). Holothurin, obtained from the water extract of these granules, consists of steroid aglycones, bound individually to four molecules of monosaccharides (Chanley, et al., 1960). Holothurin A represents 40% of the crude Holothurin and is a cholesterol-precipitated fraction (Nigrelli and Jakowska, 1960) with the empirical formula

$$C_{50\text{-}52}H_{81\text{-}85}O_{25\text{-}26}SNa$$
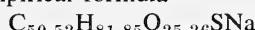
(Chanley, et al., 1959).

Infrared spectrum analysis indicates a five-or-six-membered ring lactone and one double bond. On acid hydrolysis, Holothurin A yields water-soluble aglycones, sulfuric acid and water soluble reducing sugars (Chanley, et al., 1960). Recent work has shown the presence of a half-esterified sulfate residue ($-OSO_3{}^-Na^+$), probably attached at some point in the sugar chain. Desulfation of Holothurin A occurs when treated with 0.2M methanolic HCl at 37 C. This results in a neutral product devoid of the sulfate group, while retaining unaltered glycoside-genin bonds (Friess, et al., 1967).

Investigations into properties of Holothurin have shown it to be a powerful neurotoxic agent (Freiss, et al., 1959) and to possess antitumor activity capable of suppressing growth of Sarcoma-180 (Nigrelli, 1952; Nigrelli and Zahl, 1952). Similar effects were noted on Krebs-2 ascites tumors in Swiss mice (Sullivan, et al., 1955). Hemopoietic effects have been demonstrated in *Rana pipiens* (Jakowska, et al., 1958), while hemolytic properties were noted using rabbit red blood cells (Nigrelli and Jakowska, 1960) and human erythrocytes (Thron, 1964).

In the current study, the effect of Holothurin and its fractions in a system of leucocytes displaying amoeboid movement is described. The effect of these compounds on this physiological property was interpreted in relation to surface alterations and cell membrane permeability.

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A. *Holothurin solutions*

Stock solutions of crude Holothurin, purified Holothurin A, and desulfated Holothurin A were prepared in physiological saline solution. Final concentrations are listed in Tables 1 through 11. (For Tables, see page 8 *ff.*)

B. *Leucocyte migration*

A modification of the technique developed by Ketchel and Favour (1955) was used throughout this study. Leucocyte ability to migrate by amoeboid motion was measured quantitatively as white blood cells moved away from a leucocyte buffy layer.

The technique utilized a micro-hematocrit formed inside capillary tubes each of which was approximately 7 cm long with an internal diameter of 0.8 ± 0.1 mm. New tubes were used for each experiment.

By means of capillarity, each tube was filled with heparinized blood (0.1 mg heparin/ml blood) in the presence or absence of each test compound, to two-thirds its length. One end of each tube then was heat sealed. Temperature effects associated with this method of sealing did not alter migration patterns. The tube was centrifuged at 3000 rpm for 2 minutes, producing a three component system: a lower layer of packed erythrocytes, an intermediate buffy layer of leucocytes, and an upper layer of plasma (semi-clot). Upon vertical incubation of each tube at 37°C for 5 hours, leucocytes migrated into the plasma layer. Extent of migration from the buffy coat was then measured by means of an ocular micrometer.

Trypan Blue (Tennant, 1964) staining (1% solution) was always performed on leucocytes from the buffy layer after each five hour incubation to estimate the percentage of viable cell populations and data analyzed statistically using Student's test (Snedecor, 1956).

1) *Whole blood containing Holothurin*

Hospitalized psychiatric patients, receiving no medication, served as blood donors. Two ml of heparin (2 mg/ml) in isotonic saline was added to 38 ml venous blood. This final concentration of heparin (0.1 mg/ml blood) delayed clotting. Various amounts of crude Holothurin, Holothurin A, or desulfated Holothurin A in saline or saline alone, were added to the heparinized blood, incubated for two hours at 25°C, and then used to fill each capillary tube.

2) *Leucocyte suspensions containing Holothurin*

Heparinized blood (0.1 mg heparin/ml blood) placed in long stoppered glass tubes, 6 x 250 mm, were incubated at a 45-degree angle for 45 mins.

at 37°C. The plasma-leucocyte layer was then withdrawn, pooled, and gently mixed with a pipette. Varying amounts of crude Holothurin in saline were added to the plasma suspension to attain higher concentrations than possible in whole blood, without hemolysis from erythrocytes. Incubation of the suspension porceeded for 2 hrs. at 37°C in rubber stoppered tubes (15 x 100 mm), after which the suspension was centrifuged at 1500 rpm for 5 mins. and washed once with 5-10 ml heparinized saline. After additional centrifugation at 1500 rpm for 5 mins. the saline solution was discarded and leucocytes resuspended in homologous plasma, using amounts of Holothurin-free heparinized plasma comparable to the original ratio in whole blood.

In order to use the Ketchel and Favour (1955) migration procedure, it was found necessary to have the leucocyte buffy layer overlay packed erythrocytes in each capillary tube. To obtain a leucocyte-plasma-erythrocyte ratio comparable to that which initially existed in the whole blood, heparinized blood was centrifuged in hematocrit tubes at 3000 rpm for 15 mins. From divisions on the tubes and a known total volume, it was possible to estimate the amount of each component initially present in a blood sample.

On this basis, reconstituted leucocyte-plasma-erythrocyte suspensions were placed in capillary tubes and taken through the procedure as described for whole blood. Experience has shown that leucocytes will not migrate when a buffy layer is located at the basal end of each capillary tube. Toxic factors are probably released from the glass tip during the heat sealing process.

3) *The effect of hemolysis*

a) To obtain plasma containing a high concentration of hemoglobin, several ml of heparinized blood (0.1 mg heparin/ml blood) were frozen and thawed three times using dry ice in absolute alcohol. Centrifugation at 3000 rpm for 10 minutes allowed removal of cell debris. The resulting solution consisted mainly of hemoglobin in plasma.

b) Hemolyzed blood prepared with crude Holothurin in a concentration of 20 μg/ml blood, was allowed to remain at 25°C for 1.5 and four hours. The hemolytic action of Holothurin caused a liberation of hemoglobin. After centrifugation at 3000 rpm for 10 minutes, a cell-free solution of hemoglobin remained.

To equate the intensity of liberated hemoglobin in these two plasma samples, optical density readings were made using a Klett-Summerson colorimeter (#54 filter). Heparinized blood in contact with Holothurin for 1.5

hours at 25°C and hemoglobin added to normal plasma to give a corresponding O.D., constituted solutions and readings (A). (See Table 6.) The same procedure was used for (B) solutions, except that Holothurin was allowed to remain in heparinized blood four hours at 25°C. Correspondingly more hemoglobin was then required in plasma to give higher O.D. readings in (B).

After establishing the exact amount of hemoglobin (liberated by freezing and thawing or Holothurin treatment) necessary to give comparable concentrations in whole blood, capillary tubes were filled and incubated at 37°C for five hours. Leucocyte migration was then measured by means of an ocular micrometer.

4) *Crude Holothurin versus Ouabain*

Experiments were designed to show the effect of a non-hemolytic saponin (Ouabain) in relation to a hemolytic saponin (Holothurin) on leucocyte migration.

To obtain the same molar concentration, calculations based on molecular weights of Ouabain and Holothurin were made to estimate the amount of each compound necessary to give a final concentration of 1.4×10^{-5}M in heparinized blood. Stock solutions of each compound were prepared in saline and appropriate dilutions added to heparinized blood. Capillary tubes were then filled, sealed, centrifuged, and incubated for five hours at 37°C.

5) *The influence of enzymatic inhibitors*

Freshly prepared saline solutions of the following inhibitors were added to heparinized blood and allowed to remain at 25°C for two hours: Sodium fluoride, iodoacetic acid (sodium salt), 2,4-dinitrophenol, and potassium cyanide. Concentrations are given in Tables 8, 9, 10, 11. Crude Holothurin, at a final concentration of $1 \mu g/ml$ blood was added in some cases. All quantities of inhibitors and Holothurin in blood were of volumes less than one part inhibitor or Holothurin to four parts of heparinized blood. Saline controls always contained the same volume ratio. After filling, sealing, and centrifuging (3000 rpm for two minutes) each capillary tube was incubated in a vertical position for five hours at 37°C.

RESULTS

In all cases with crude Holothurin, Holothurin A or desulfated Holothurin A, low concentrations showed a significant increase in the rate of leucocyte amoeboid motion. The stimulation by crude Holothurin (Table 1) occurred mainly in the range of 0.1 to 4 $\mu g/ml$ blood. Inhibition by crude Holothurin (Table 2) resulted in a decrease in leucocyte migration,

principally at levels between 16 and 22 μg crude Holothurin/ml blood. Trypan Blue staining showed the presence of viable cells in all experiments. Effects were not due to massive alterations in the test system; however, considerable hemolysis was noted in tubes treated with higher concentrations of Holothurin. No significant results were observed in concentrations ranging from 1 to 14 $\mu g/ml$.

Holothurin A was found to be effective in lesser amounts, producing a stimulation at a concentration of 0.02 $\mu g/ml$ and a decrease in the rate of amoeboid motion at a concentration of $0.1\mu g/ml$. Movement of leucocytes was completely inhibited at a concentration of 20 $\mu g/ml$, with the subsequent liberation of inhibitory concentrations of hemoglobin from erythrocytes.

When desulfated Holothurin A is compared to crude Holothurin and Holothurin A it will be noted that the biological activity of this compound has been greatly changed. Thus, a stimulation in migration will occur at $1\mu g/ml$, while concentrations greater than 100 $\mu g/ml$ were necessary to inhibit leucocyte motion.

Observations indicate that the hemolytic properties of crude Holothurin in higher concentrations may have some effect on leucocytes in whole blood (Table 5). A crude Holothurin concentration of 40 $\mu g/ml$ leucocyte suspension ¨(23 $\mu g/ml$ blood) does not significantly alter the rate of white blood cell migration, however concentrations from 100-1000 $\mu g/ml$ leucocyte suspension (greater than 58 $\mu g/ml$ blood) will significantly decrease the rate of motion. A significant alteration in cell viability occurred in the presence of higher concentrations of Holothurin when levels greater than 100 $\mu g/ml$ leucocyte suspension were reached.

To substantiate the fact that crude Holothurin *per se* had affected leucocyte mobility, experiments were designed to compare results obtained when the non-hemolytic saponin Ouabain was used in the same concentration as the hemolytic saponin (crude Holothurin). Results showed no significant differences between the two saponins at a concentration of 1.4×10^{-5}M (16 $\mu g/ml$ blood). (Table 8.) The data indicate approximately the same decrease in migration when compared with the control. Trypan Blue staining demonstrated the viable nature of these cells within an acceptable range. No gross mortality had caused alterations in the rate of leucocyte migration.

Holothurin is known to possess strong hemolytic properties with concentrations as linear functions of the red blood cell count and reaction time (Thron, 1964). The effect of homologous hemoglobin, in the absence of crude Holo-

thurin, was found to affect leucocyte migration only in concentrations (O.D.) comparable to that produced by crude Holothurin (O.D. 250) in contact with red blood cells for four hours at 25°C. The hemoglobin concentration (O.D. 103) comparable to crude Holothurin hemolysis after contact for 1.5 hours did not affect leucocyte migration. It appears that the amount of hemoglobin liberated by crude Holothurin after four hours will significantly decrease the rate of leucocyte motion (Table 6).

The effect of selected inhibitors on white cell migration was studied with the hope of obtaining information on metabolic pathways in the presence and absence of crude Holothurin. Both anaerobic inhibitors (sodium fluoride and iodo-acetate) depressed migration in concentrations less than $1 \times 10^{-3}M$, while the aerobic inhibitors (2,4-dinitrophenol and potassium cyanide) required greater amounts ($1 \times 10^{-2}M$ or more) to produce a decrease. In the case of dinitrophenol, solubility problems prevented an accurate estimation of the amount required to produce an inhibition.

A study of inhibitors, in the presence of crude Holothurin, was undertaken in an attempt to explain the stimulation of migration resulting from use of low concentrations ($8.8 \times 10^{-7}M$ or $1 \mu g/ml$ blood) of this compound. In all cases with both aerobic and anaerobic inhibitors, a decrease in migration occurred in the presence of crude Holothurin. When compared to the rate of leucocyte mobility in the absence of inhibitors, it is possible to state that crude Holothurin stimulation is the result of both aerobic and anaerobic metabolism. Leucocyte non-viability was not a factor since Trypan Blue staining indicated a cell survival greater than 95%. All concentrations used allowed each experiment to be carried out under optimal conditions.

Discussion

The biological and chemical activities of Holothurin put this compound in the class of steroid saponins (Nigrelli and Jakowska, 1960) with surface-active properties (Seeman, 1967; Thron, 1964) and ability to alter cell membrane structure. High surface activity of echinoderm toxins, structural arrangement, and chemical properties contribute to their ability to penetrate membranes and demonstrate an affinity for cellular components (Ruggieri, 1965). These properties are significant in the study of Holothurin action on leucocytes in relation to systems incorporating surface phenomena, such as amoeboid movement.

By definition (White, *et al.*, 1964), all saponins lower surface tensions and contribute to

cytolytic effects when used in sufficient quantity. The work of Ponder and McLeod (1936) has shown that saponin hemolytic substances will affect white cells in a manner similar to their effect on red cells. Current experimental data using suspensions of leucocytes confirms these earlier reports. Crude Holothurin in concentrations greater than 100 $\mu g/ml$ will cause a certain percentage of white cell disintegration, decreased cell counts, and altered cell viability.

Since Holothurin is chemically a member of the saponin family, it is expected that this compound will show activity towards cell membranes. Studies by Friess, *et al.*, (1960) postulate that the Holothurin effect may be a non-specific surface action on cell membranes or that it could be a specific attack at one or more sites in the membrane. The demonstration that Holothurin can produce cytotoxic effects on leucocytes, as shown by Trypan Blue uptake and altered morphology in higher concentrations, would suggest that Holothurin *in vitro*, and possibility *in vivo*, can affect integrity and permeability of this particular cell type. The migration system had advantages over others since it utilized a buffy layer of leucocytes from which isolated cells could move away from the main population.

It has been suggested (Parpart and Ballentine, 1952) that cell membrane structure is a mosaic of cylinders containing phospholipid and cholesterol surrounded by a protein meshwork. Observations by Dourmashkin, *et al.*, (1962) have shown that, in the presence of suitable agents, extensive re-arrangement of these membrane lipids may occur. The original membrane is rendered more permeable by incorporation of saponin, thereby forming a more permeable structure with spaces 90 Angstroms across that appear to contain water. Saponin hemolytic activity is thought to depend on these spaces in the lipid component of cell membranes. Alterations in lipid structures of this kind are important in controlling the permeability of cells under physiological conditions. It is therefore suggested, that changes in leucocyte permeability in the presence of Holothurin could be responsible for several effects observed during the current study with this compound. Holothurin is known to be a surface active agent and it is suspected that a change in cell permeability and eventual destruction of Holothurin-treated leucocytes (in concentrations greater than 100 $\mu g/ml$) are initiated by reactions on the cell surface. In addition, Holothurin may alter cell metabolism by virtue of its action at cell surfaces to allow freer passage of this compound to an intracellular environment.

Treatment of human leucocytes with crude Holothurin, Holothurin A, or desulfated Holothurin A resulted in a stimulation of migration as well as inhibition of phagocytic motility. Concentrations were chosen which could affect these physiological properties but not cause cell death in most cases. Results may be explained on the basis of structure, surface properties, or by consideration of the role of chemical interaction between a surface-active agent (Holothurin) and the cells involved. Chemical approaches to amoeboid movement have been lacking; however, it is suspected that Holothurin may enhance migration through a stimulation of leucocyte glycolysis. It is well established that certain substances, as bacterial endotoxins, hormones, and polysaccharides (Woods *et al.,* 1961), produce a glycolytic stimulation and that this stimulation can be associated with an increase in functional capacities of the cells.

On the basis of chemical structure, it will be noted that the concentration of desulfated Holothurin A required to produce a decrease in migration was considerably higher (greater than 100 μg/ml) than that necessary to inhibit leucocyte mobility in the presence of crude Holothurin or Holothurin A. Certain structural configurations of Holothurin modify leucocyte motility in systems involving physiological response. The action of a saponin devoid of the acidic sulfuric acid group may be interpreted in view of its ability to combine with basic groups of membrane proteins. It has been noted by Friess, *et al.,* (1967) that the anionic nature of Holothurin facilitates permeability through membranes, allowing greater speed of action and therefore a more complete reaction in a given time. Desulfated Holothurin A demonstrates an impeded degree of membrane permeation due to a separation of lipid and polysaccharide tissue phases. Toxic manifestations accordingly would be inhibited during the initial phases of interaction within cells. Friess, *et al.,* (1967), also note that a resulfation of desulfated Holothurin may be necessary for this compound to become biologically active.

An alternative approach to explain the stimulation of leucocyte migration resides in an area related to the lytic properties of Holothurin. Holothurin has a strong hemolytic potency since it has a high affinity for erythrocytes. In suspensions of heparinized blood, where both erythrocytes and leucocytes were present, low concentrations of Holothurin were probably taken up by red cells, leaving little or no free lysin to act on the white cells. More Holothurin caused greater absorption by red cells at a constant number; however more free lysin also remained in solution to act on the leucocytes. Under these conditions a stimulation of leucocyte migration is merely the rate occurring where extremely small amounts of Holothurin have remained free in the system (less than 0.02-4 μg/ml). Eventually a concentration of free Holothurin is reached that will be too great to cause a stimulation but not sufficient to produce an inhibition. This will occur in a suspension of blood initially receiving 6-14 μg of crude Holothurin/ml blood, and is referred to as the intermediate range. In crude Holothurin concentrations initially greater than 16 μg/ml blood, much free saponin will be available to inhibit leucocyte activity. As the concentration of this compound is further increased, results (Table 5) indicate much cell destruction concomitant with a significant decrease in leucocyte mobility and the presence of numerous non-viable cells. On microscopic examination, distortion of cell morphology, altered staining properties and fragmentation were evident. Macroscopically at a concentration of 580 μg crude Holothurin/ml blood, viscous turbid solutions of leucocyte debris resulted after approximately one hour in the presence of this compound. It is obvious that cell integrity was being altered, membranes were being attacked, and leucocyte structure was being destroyed.

The effect of liberated hemoglobin also was studied in the migration system. Recent studies by Rideal & Taylor (1958) support the view that hemolysis, caused by saponins, involves adsorption of hemolytic agents on the cell wall. This adsorption alters bound cholesterol, with eventual cell wall destruction and release of hemoglobin. Rate of liberation is dependent on the concentration of saponin present in the system, and a period of time is required for all hemoglobin to be released from the erythrocytes. Table 6 shows the effect of hemoglobin on leucocyte migration. Low concentrations of hemoglobin do not alter migration patterns whereas concentrations attained after four hours, using a crude Holothurin concentration of 20 μg/ml, seem to display an inhibitory effect. Additional studies with leucocyte suspensions (Table 5) indicate that free hemoglobin has been a factor to depress migration in crude Holothurin concentrations of approximately 20 μg/ml blood (Table 2). To further substantiate this conclusion, the non-hemolytic saponin Ouabain was used in a system of heparinized blood (Table 7) at a lesser concentration (1.4 x 10^{-5}M). Migration results at this level of saponin are due to an effect of the compound and not to the presence of hemoglobin, assuming its action on cell membranes and biochemical processes is similar to that of crude Holothurin. No significant differences were noted between crude Holothurin

and Ouabain when used in the same concentration (Table 7). Both saponins were able to depress significantly leucocyte migration when compared to saline controls.

Several inhibitors were studied in the migration system to further elucidate factors involved in leucocyte metabolism in the presence of Holothurin. A decrease in migration may be explained as the result of inhibition of either glycolytic or oxidative respiratory metabolism of leucocytes. In present studies two anaerobic inhibitors, iodoacetate (8×10^{-5}M) and sodium fluoride (1×10^{-3}M), were found to be most effective towards inhibiting leucocyte mobility. The aerobic inhibitors, 2,4-dinitrophenol and potassium cyanide, were not inhibitory until concentrations greater than 1×10^{-2}M were reached.

The fact that leucocyte metabolism is mainly glycolytic and mobility is inhibited by glycolytic inhibitors would imply that the energy provided by glycolysis is a factor in amoeboid movement. The use of greater amounts of aerobic inhibitors, noted in current studies, would further suggest that aerobic systems affected by cyanide and dinitrophenol also contribute to the production of energy.

Crude Holothurin in a concentration of 1 μg/ml used concomitantly with these inhibitors showed the same migration patterns as inhibitors in the absence of Holothurin. However, a stimulation resulting from a low concentration of crude Holothurin, was evident in all experiments where this saponin was used in the absence of inhibiting compounds. The stimulation of leucocyte migration in the presence of crude Holothurin at low concentrations (1 μg/ml) was due to both aerobic and anaerobic metabolism.

Summary

1) A stimulation of leucocyte migration occurred in the concentration range 0.1-6 μg crude Holothurin/ml blood, at 0.02 μg Holothurin A/ml blood and 1.0 μg desulfated Holothurin A/ml blood.

2) An inhibition of leucocyte migration was produced in concentrations of 16-58 μg crude Holothurin/ml blood, at 0.1 μg Holothurin A/ml blood and 300 μg desulfated Holothurin A/ml blood.

3) Ouabain, a non-hemolytic saponin, inhibited leucocyte migration to the same extent as crude Holothurin when used in a concentration of 1.4×10^{-5}M. (16 μg/ml).

4) Liberated hemoglobin was found to be inhibitory in high concentrations, while lesser amounts did not alter white cell mobility.

5) Crude Holothurin (1.0 μg/ml blood) used concomitantly with anaerobic inhibitors NaF and iodoacetic acid and with aerobic inhibitors 2,4-dinitrophenol and KCN, showed no alteration in migration patterns different from that obtained in the absence of this saponin. Both aerobic and anaerobic metabolism are important for the stimulation of leucocyte migration in the presence of low concentrations of crude Holothurin.

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TABLE 1. CRUDE HOLOTHURIN STIMULATION OF LEUCOCYTE MIGRATION USING WHOLE BLOOD

Concentration of Holothurin in μg/ml	Holothurin + leucocytes (migration in mm)	Saline* + leucocytes (migration in mm)	Per cent viable Holothurin- treated leucocytes	N	P
0.1	1.94	1.63	100	30	< .001
	2.55	2.38	—	30	< .001
	2.49	2.11	—	30	< .01
	1.88	1.79	—	30	< .1
0.5	2.68	2.11	—	30	< .001
	2.02	1.63	100	30	< .001
	2.22	2.10	—	30	< .001
	2.61	2.38	—	30	< .001
	1.96	1.79	—	30	< .01
1.0	1.97	1.63	99-100	30	< .001
	2.01	1.79	—	30	< .001
	1.50	1.34	—	30	< .001
	2.78	2.11	—	30	< .001
	1.98	1.79	—	30	< .001
	1.89	1.84	—	30	< .3
2.0	2.09	1.63	—	30	< .001
	1.47	1.34	—	30	< .001
	1.81	1.79	—	30	< .8
	1.82	1.79	—	30	< .6
3.0	2.18	1.63	—	30	< .001
4.0	1.90	1.79	99-100	30	< .02
	2.16	2.02	—	30	< .1
6.0	1.84	1.79	—	30	< .5

* Control cells always showed 95-100% viability.

TABLE 2. CRUDE HOLOTHURIN INHIBITION OF LEUCOCYTE MIGRATION USING WHOLE BLOOD

Concentration of Holothurin in μg/ml	Holothurin + leucocytes (migration in mm)	Saline* + leucocytes (migration in mm)	Per cent viable Holothurin- treated leucocytes	N	P
0.1	1.72	1.84	—	30	< .01
0.5	1.79	1.84	—	30	< .2
1.0	2.34	2.38	—	30	< .3
	1.77	1.79	99-100	30	< .8
2.0	2.09	2.38	—	30	< .001
4.0	1.73	1.76	—	30	< .7
	1.74	1.79	99-100	30	< .6
6.0	2.08	2.08	100	30	< .1
	1.20	1.30	—	30	< .2
8.0	1.19	1.30	97	30	< .4
	1.77	1.79	—	30	< .9
10.0	1.18	1.30	97-100	30	< .3
12.0	1.19	1.30	90	30	< .2
14.0	1.18	1.30	95	30	< .2
16.0	1.52	2.12	90	30	< .001
	1.06	1.30	90-95	30	< .02
18.0	0.66	1.30	90	30	< .001
20.0	0.70	1.30	90-95	30	< .001
22.0	0.51	1.30	90	30	< .001

* Control cells always showed 95-100% viability.

TABLE 3. THE EFFECT OF HOLOTHURIN A ON LEUCOCYTE MIGRATION USING WHOLE BLOOD

Concentration of Holo- thurin A in µg/ml	Holothurin A + leucocytes (migration in mm)	Saline* + leucocytes (migration in mm)	Per cent viable leucocytes	N	P
20**	none	1.62	98-100	28	—
0.1	1.53	1.80	99-100	20	< .001
0.025	1.46	1.51	99-100	23	< .05

* Control cells showed 100% viability.
** Much hemolysis present.

TABLE 4. THE EFFECT OF DESULFATED HOLOTHURIN A ON LEUCOCYTE MIGRATION USING WHOLE BLOOD

Concentration of desulfated Holothurin A in µg/ml	Desulfated Holothurin A + leucocytes (migration in mm)	Saline* + leucocytes (migration in mm)	Per cent viable leucocytes	N	P
1	1.24	1.70	100	33	< .01
20	1.69	1.62	100	28	< .8
100	1.38	1.43	100	34	< .4
300	0.68**	1.42	70	7	< .001

* Control cells showed 100% viability.
** Slight amount of hemolysis.

TABLE 5. CRUDE HOLOTHURIN INHIBITION OF LEUCOCYTE MIGRATION
USING WHITE BLOOD CELL SUSPENSIONS

Holothurin concentration in WBC sus- pension, µg/ml leucocytes	Comparable Holothurin concentration in whole blood, µg/ml whole blood	Holothurin- treated leucocytes (migration in mm)	Saline-treated leucocytes* (migration in mm)	Per cent viable Holothurin- treated leucocytes	N	P
40	23	1.54	1.67	99-100	15	< .2
100	58	0.90	1.67	85	20	< .001
300	174	0.66	1.67	30-50	14	< .001
1000	580	0.49**	1.67	5***	—	< .001

* Control cells showed 99-100% viability.
** Few cells.
*** Most cells disintegrated.

TABLE 6. THE EFFECT OF HEMOGLOBIN ON LEUCOCYTE MIGRATION

Compound	Lapsed time of contact	Klett O.D.	Average migration (in mm)	Per cent viable	N	P
Crude Holothurin (20 μg/ml)	1.5 hrs.	107	1.50	100	29	
Hemoglobin (A)	—	103	2.44	100	20	< .8
Saline	—	—	2.42	100	34	
Crude Holothurin (20 μg/ml)	4.0 hrs.	250	1.37	99	25	
Hemoglobin (B)	—	270	1.97	100	20	< .001
Saline	—	—	2.42	100	34	

TABLE 7. LEUCOCYTE MIGRATION IN THE PRESENCE OF CRUDE HOLOTHURIN AND OUABAIN

Compound	Concentration	Average migration (in mm)	Per cent viable	N	P
Crude Holothurin	1.4×10^{-5}M	1.52	90	20	
Ouabain	1.4×10^{-5}M	1.44	92-95	31	< .5
Saline	—	2.12	95-100	28	

TABLE 8. LEUCOCYTE MIGRATION IN THE PRESENCE OF CRUDE HOLOTHURIN AND SODIUM FLUORIDE

Compound	Concentration	Average migration (in mm)	Per cent viable	N
1) Holothurin (1 μg/ml)	8.8×10^{-7}M	1.86	99-100	25
2) Sodium fluoride	8.0×10^{-5}M	1.65	98-99	31
3) Holothurin (8.8×10^{-7}M) + NaF	8.0×10^{-5}M	1.68	100	29
4) Holothurin (8.8×10^{-7}M) + NaF	1.0×10^{-1}M	1.61	99	30
5) Holothurin (8.8×10^{-7}M) + NaF	1.0×10^{-3}M	1.03	98-99	28
6) Saline	—	1.63	98	28
7) Sodium fluoride	8.8×10^{-7}M	1.72	99	25
8) Sodium fluoride	1.0×10^{-3}M	1.10	99-100	28
9) Saline	—	1.73	99-100	28

1) versus 6); P < .001

2) versus 6); P < .6

3) versus 6); P < .3

4) versus 6); P < .7

5) versus 6); P < .001

7) versus 9); P < .7

8) versus 9); P < .001

TABLE 9. LEUCOCYTE MIGRATION IN THE PRESENCE OF CRUDE HOLOTHURIN AND IODOACETIC ACID

Compound	Concentration	Average migration (in mm)	Per cent viable	N
1) Holothurin (1 μg/ml)	$8.8 \times 10^{-7}M$	1.82	98-100	32
2) Iodoacetic acid	$8.0 \times 10^{-5}M$	0.98	100	31
3) Holothurin ($8.8 \times 10^{-7}M$) + IAA	$8.0 \times 10^{-5}M$	0.91	97-100	31
4) Saline	—	1.66	99-100	31
5) Iodoacetic acid	$8.8 \times 10^{-7}M$	1.65	100	20
6) Iodoacetic acid	$1.0 \times 10^{-4}M$	0.26	98-100	20
7) Saline	—	1.53	99-100	18

1) versus 4); $P < .001$
2) versus 3); $P < .3$
2) versus 4); $P < .001$
3) versus 4); $P < .001$
5) versus 7); $P < .2$
6) versus 7); $P < .001$

TABLE 10. LEUCOCYTE MIGRATION IN THE PRESENCE OF CRUDE HOLOTHURIN AND DINITROPHENOL

Compound	Concentration	Average migration (in mm)	Per cent viable	N
1) Holothurin	$8.8 \times 10^{-7}M$	1.90	99-100	35
2) Dinitrophenol	$8.0 \times 10^{-5}M$	1.65	100	35
3) Holothurin ($8.8 \times 10^{-7}M$) + DNP	$8.0 \times 10^{-5}M$	1.78	100	34
4) Saline	—	1.73	100	36
5) Dinitrophenol	$1.0 \times 10^{-3}M$	1.64	100	26
6) Saline	—	1.60	100	22
7) Dinitrophenol	$1.0 \times 10^{-4}M$	1.50	95	20
8) Dinitrophenol	$8.8 \times 10^{-7}M$	1.55	100	15
9) Saline	—	1.50	99-100	25

1) versus 4); $P < .001$
1) versus 3); $P < .02$
2) versus 4); $P < .02$
3) versus 4); $P < .4$
5) versus 6); $P < .6$
8) versus 9); $P < .7$

TABLE 11. LEUCOCYTE MIGRATION IN THE PRESENCE OF CRUDE HOLOTHURIN AND POTASSIUM CYANIDE

Compound	Concentration	Average migration (in mm)	Per cent viable	N
1) Holothurin	$8.8 \times 10^{-7}M$	1.79	98-100	31
2) Potassium cyanide	$8.0 \times 10^{-5}M$	1.54	99-100	35
3) Holothurin ($8.8 \times 10^{-7}M$) + KCN	$8.0 \times 10^{-5}M$	1.50	98	33
4) Saline	—	1.50	99-100	33
5) Potassium cyanide	$1.0 \times 10^{-4}M$	1.60	99	27
6) Potassium cyanide	$1.0 \times 10^{-3}M$	1.66	98-99	29
7) Potassium cyanide	$1.0 \times 10^{-2}M$	0.76	99-100	27
8) Saline	—	1.60	100	25
9) Potassium cyanide	$8.8 \times 10^{-7}M$	2.14	99-100	30
10) Saline	—	2.14	100	27

1) versus 4); $P < .001$

2) versus 4); $P < .5$

6) versus 8); $P < .7$

7) versus 8); $P < .001$

NEWS AND NOTES

New York Medical College to Sponsor Cooperative Program in Comparative Pathology

A group of scientists at New York Medical College believes that a major contribution to mankind can be made from veterinary medicine within the next decade, and has instituted a cooperative program with local zoos in which a flow of information on spontaneously occurring animal diseases can be applied to the human health sciences. The Department of Pathology, through its chairman, David Spiro, M.D., Ph.D., has announced the formation of a Comparative Pathology Program in which animal disease models which have human counterparts will be studied in depth.

Man shares many diseases with his non-human brothers and the program's Directors, Ralph E. Strebel, Ph.D., associate professor of pathology, Edward Garner, D.V.M., assistant professor of pathology, and Emil Dolensek, D.V.M., veterinarian of the Bronx Zoo and assistant professor of comparative pathology at New York Medical College, believe that studies of these animals could shed light on many aspects of human pathology.

In order to provide future practitioners and research pathologists with a broad-based approach to the pathogenesis of disease while also meeting the need for improved animal health management, the investigators plan to utilize material from a wide variety of wild, domestic, and zoo animals.

The program functions as part of a cooperative interprofessional arrangement with the New York Zoological Society, through its director, William G. Conway, and Dr. Dolensek. Other cooperating institutions include the Staten Island Zoo, which is operated by the Staten Island Zoological Society, and the Prospect Park, Flushing, and Central Park zoos, which are under the jurisdiction of New York City's Parks, Recreation, and Cultural Affairs Administration, They are represented respectively by William Summerville, General Curator; Ronald Ellis, Supervisor;

Mrs. Gilette Infante, Supervisor; and John Fitzgerald, Supervisor.

Calling material from these zoo populations a "vast unexplored reservoir of valuable disease models," Dr. Strebel and Dr. Garner believe that material thus obtained will become a unique resource for the study of such conditions as arteriosclerosis, cancer, heart disease, diabetes, hepatitis, and many other diseases shared by man with animals. To expedite the search for animal diseases which might prove fruitful in comparative studies, the program will also call upon the services of two pathologists experienced in human disease, Dr. Henry I. Kobrin of New York Medical College, and Dr. John Budinger, consultant to the New York Zoological Society.

Profiles of Normal Values Sought

The medical treatment of zoo animals is hampered presently because of a dearth of information on what constitutes the norm for blood values in all orders of zoo animals. Therefore, efforts will be made to develop comprehensive normal blood profiles for zoo animal populations. This should greatly enhance future attempts to compare normal animals of different orders and species as well as to establish normal base lines necessary for the appropriate treatment of their diseases.

Registry of Animal Diseases

The information which will be derived from diagnostic and necropsy procedures will be utilized to develop a comprehensive registry of animal diseases. When completed, this registry will provide valuable reference material to investigators, medical students, and graduate students studying comparative pathology.

Graduate Degree Program Offered

New York Medical College will offer qualified individuals a graduate degree program in com-

parative pathology. The unique aspect of this program will be the depth of exposure to an exceptionally broad spectrum of animal as well as human pathology. In addition to comprehensive course work and enrollment in the human pathology program offered at New York Medical College, students will spend several months at the various zoos on a rotation basis. The broad scope of this orientation will afford the student an exposure to the pathogenesis of disease on a comparative basis in a wide variety of animal life. The combination of excellent training in both animal and human pathology is a rarity. A person, so exposed, would be in a superior position to make effective use of animal pathology and animal models of disease for purposes of study and research in regard to the pathogenesis of human disease.

NOTICE TO CONTRIBUTORS

Manuscripts must conform with the Style Manual for Biological Journals (American Institute of Biological Sciences). All material must be typewritten, double-spaced, with wide margins. Papers submitted on erasable bond or mimeograph bond paper will not be accepted for publication. Papers and illustrations must be submitted in duplicate, and the editors reserve the right to keep one copy of the manuscript of a published paper.

The senior author will be furnished first galley proofs, edited manuscript, and first illustration proofs, which must all be returned to the editor within three weeks of the date on which it was mailed to the author. Papers for which proofs are not returned within that time will be deemed without printing or editing error and will be scheduled for publication. Changes made after that time will be charged to the author. Author should check proofs against the edited manuscript and corrections should be marked on the proofs. The edited manuscript should not be marked. Galley proofs will be sent to additional authors if requested. Original illustrative material will be returned to the author after publication.

An abstract of no more than 200 words should accompany the paper.

Fifty reprints will be furnished the senior author free of charge. Additional reprints may be ordered; forms will be sent with page proofs.

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Species Identification of Commercial Crocodilian Skins

F. Wayne King[1] and Peter Brazaitis[2]

(Figures 1-41)

Gross similarities in the morphology of crocodilian skins has made specific identification of individual commercial hides extremely difficult. Qualitative and quantitative differences between the hides of various species are defined and form the basis of a key to commercial crocodilian hides. The distribution, common, and commercial names, distinguishing characteristics of the hides, and the status of the wild populations of each of the 27 species and subspecies is given.

Introduction

To the layman most crocodilians look similar — all have relatively long toothy snouts, scaly backs, flattened oar-like tails, large webbed hindfeet, and most have crossbanded color patterns. As a consequence, most Americans mentally lump all crocodilians under the collective heading "alligator." Profound differences exist between the species of crocodilians, but the untrained eye notices the gross similarities rather than the less obvious differences. When it comes to identifying a species of crocodilian from a commercial hide, however, even a trained herpetologist faces serious difficulty. All commercial skins are grossly alike. All crocodilian leather is retailed throughout the United States as "alligator," while in Europe, Africa, and Asia the same hides are sold as "crocodile."

In this paper, we attempt to provide means to identify commercial crocodilian hides. Since the paper will be read by layman, trained herpetologist, government inspector, and commercial dealer alike, we have endeavored to use terminology comprehensible to all. Where there is a chance of confusion, we have provided photographs and line drawings for clarification.

Materials and Methods

Comparisons were made between the skins of live specimens in zoos and private collections, preserved specimens and dried skins in museum collections, and tanned and finished commercial skins supplied by the Reptile Products Association of the United States. A total of over 350 specimens were examined. Museum specimens of every species and subspecies were studied. Living specimens of every form, except *Caiman crocodilus apaporiensis* and *Crocodylus siamensis*, were seen. Commercial hides of most species were examined. The notable exceptions were *Gavialis gangeticus*, *Alligator sinensis*, *Paleosuchus palpebrosus*, *Paleosuchus trigonatus*, *Crocodylus palustris*, and *Crocodylus rhombifer*. The raw data are on deposit at the New York Zoological Park. The characters and terms used in this study are defined below.

COMMERCIAL HIDES. Hides used in the crocodilian hide trade for the manufacture of leather goods are termed commercial hides, whether they are raw skins or are in the process of being tanned and finished.

HORNBACK HIDES. Rough dorsal (back) skins obtained by skinning the animals beginning from an incision made along the midventral (belly) line. Large bony dorsal scales, usually with raised keels, occupy the center of the hide. Smooth squarish scales from the ventral surface are located along the lateral edges of the hide. Hornback hides usually are skinned from relatively small specimens since the heavily ossified dorsal scales of adults make their hides stiff and limits its use for leather. Skin from the tail and proximal portion of the legs is attached to the hide (figure 1).

BELLY HIDES. Smooth ventral (belly) skins obtained by skinning the animal beginning at an incision just below the large bony dorsal scales high on one side and continuing down the side, under the body, and up the other side to the

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edge of the large dorsal scales on that side of the back. The scales of belly skins are squarish or rectangular in shape over most of the center of the skin. The round or oval scales from the side of the body are located along the lateral edges of the hide. The vent is represented by a hole along the midline of the skin. Skin from the tail and proximal portion of the legs is attached (figure 1).

BUTTON-BELLY AND SOFT-BELLY HIDES. Belly skins that possess osteoderms, or buttons (the commercial name), are called button hides. Belly skins that lack osteoderms are called soft-belly hides. Button-belly hides will not flex through the middle of a scale because of the osteoderm button. Soft-belly hides not only flex between scales but also bend to a lesser degree through the middle of scales (figure 2). As a result the most sought after hides are soft-bellies. Button-bellies with large osteoderms are most frequently used for making the flat, non-flexing sides of purses or attaché cases. The stiffness of these skins limits their use where flexibility is demanded, as in shoes, belts, and watch-bands.

SIDES. A narrow strip of soft skin taken from a point under the lower jaw and extending back over the front leg, along the side of the trunk, under the rear leg and ending near the vent (figure 15). Such strips are skinned from large caimans (*Caiman, Melanosuchus, Paleosuchus*) which have such heavy osteoderm buttons in the belly skin, as to make this skin almost worthless as leather. The hide-hunters avoid both the bony dorsal scales and the bony belly scales by removing only the soft side skin. The scales on sides consist of large, round to diamond-shaped scales separated by soft skin or small scales. The largest scales usually possess osteoderm buttons and may have a low keel.

Sides could, of course, be cut from soft-bellied alligators and crocodiles, as well as button-bellied caimans. The reason they are not is that soft-belly hides are worth more with the sides attached than they are with the strips removed. The best tanned soft-belly hides sell for as much as $12.00 per square foot. Tanned sides sell for only $5.00 to $15.00 apiece.

THROATS, SIDES, AND GIRDLES. Throats are V-shaped pieces skinned from under the chin and the sides of the neck. Girdles are taken from the thighs and belly immediately anterior to the vent. Sides, which accompany throats and girdles, are stripped from the sides of the trunk between the legs (figure 20). These cuts of skin are from exceptionally large caimans (*Melanosuchus*, and possibly *Caiman*)

which have heavy bony belly osteoderms. The large size of the scales on these pieces attests to the size of the animals they come from.

FLIPPERS. The small, irregular-shaped pieces of skin from the legs. The scales are usually uniformly small, smooth, and squarish.

RAW HIDES OR SALTED HIDES. Untanned commercial hides. They may be dry or moist from the salt. They usually are rolled up for shipment, and retain the color pattern of the live specimen — most frequently dark spots or dark crossbands on the side of the trunk and tail.

CRUSTS. Hides which have been tanned, but not dyed or polished. Crusts are usually ash gray or tan, and have a dull, unpolished finish (figures 9, 10, and 15). The next step in the finishing process is to bleach or dye the hide its final color. If it is a button-belly, the osteoderms are shaved from the inside of the hide to eliminate as much of them as possible. It is not possible to remove every osteoderm button in its entirety, but it is possible to remove enough of them to make a stiff hide much more flexible (figures 14 and 19). Unskilled tanners may shave the hide too closely and leave thin, weak sections between the rows of scales.

POLISHED AND FINISHED HIDES. After dyeing, skins customarily are given a high-gloss finish by burnishing the scales under the pressure of a polishing wheel. The glossy finish is characteristic of most crocodilian products. After the hide has been finished, it is ready for cutting into the pieces that are to be made into the manufactured product.

FLAT FINISH AND BOMBE FINISH. Finished skins with flat, level scales are flat finished skins. Those in which the individual scales are slightly curved, with the center of each one arching up from the crease where it meets adjacent scales, are given the French name *bombe*.

SAUVAGE FINISH. Not all finished hides have a highly polished surface. Some are finished with a process that retains much of the texture of the original crust. The result is a textured, non-glossy, oiled-leather appearance called *sauvage* (figure 9).

VENTRAL SCALES AND LATERAL SCALES. Scales from the underside of the throat, body, and tail are ventral scales (figure 1). They are large and squarish in all crocodilians. The individual square scales are in contact with adjacent scales and are arranged in rows across the belly of the animal. Scales from the side of the body are lateral scales (figure 1).

They frequently are arrayed in two distinct size classes, the larger composed of oval and diamond-shaped scales. These individual scales usually are not in contact with adjacent scales.

Ventral and lateral scales possess a number of characteristics which may be used to identify a particular species or group of species. Most commercial skins used in the United States are either belly skins or sides, and since even hornback hides normally retain ventral scales along their edges, only the ventral and lateral scales are considered in this paper.

BUTTONS. The bony osteoderms present in the scales of many crocodilians. The presence of buttons in the ventral scales of belly skins gives them their commercial name, button-belly hides. Buttons can be seen if the hide is turned over and the backside (inside) examined. Even though they are decalcified during tanning, the osteoderm buttons are a different color. In crusts and light-colored finished skins, buttons are usually slightly darker than the rest of the skin (figures 13 and 14). In dark finished skins, they may be lighter than the surrounding tissue, although they are usually darker (figures 19, 34, 35, 36, and 37).

In many cases buttons also can be detected from the front side (outside) of a finished skin as ill-defined light-colored blotches in the center of the ventral scales (figures 18 and 34). They may also be evident as raised or slightly sunken areas on the surface of the ventral scales. They can best be seen by holding the polished surface of the scale in a position where light reflected off its surface reaches your eye. In this position any surface irregularity becomes apparent (figures 35 and 41).

SURFACE PITTING. During the tanning process, the bony osteoderms do not shrink as much as the non-calcified tissue. As a result, button skins may exhibit some of the pockmarked or wrinkled texture of the underlying osteoderms. This condition is called surface pitting. Surface pitting is best seen where the osteoderms are most heavy, as in the dorsal scales of hornback hides, and the ventral scales of the skins of caiman (*Caiman, Melanosuchus, Paleosuchus*), slender-snouted crocodile (*Crocodylus cataphractus*), dwarf crocodile (*Osteolaemus*), or button-belly Nile crocodile (*Crocodylus niloticus*) (figures 10, 11, 12, 18, 36, and 37).

The skins of some species are more strongly pitted than others; adult specimens tend to be more pitted than juveniles; and not all individuals exhibit the same degree of pitting throughout all their scales.

SINGLE BUTTONS AND DOUBLE BUTTONS. Those crocodile and alligator specimens which have osteoderms are characterized by having single buttons, one osteoderm button per scale (figures 8, 34, 36, and 37), while all caimans have double buttons, two osteoderms per scale (figures 13, 14, and 19). In caimans, the posterior of the two buttons occupies almost the entire area of the scale. The anterior button occupies the anterior one-quarter of the scale and curves upward and inward (mesially) to protectively overlap the posterior margin of the next anterior scale and the intervening soft skin. The overlapping of osteoderms is an evolutionary adaptation that affords more protection to the weak hinge point between the scales, although it causes the loss of some freedom of movement. The dwarf caimans (*Paleosuchus*), with proportionally the largest double buttons of any species of crocodilian, are well on the way to evolving the stiff analog of a turtle plastron. The two parts of the caiman double button are more evident before the buttons are shaved during finishing (figure 14). During shaving, the anterior, inward-curving button is nearly completely removed. In most cases, however, part of the anterior button remains even after the shaving is complete (figure 19).

FOLLICLE GLAND. Belly scales of gavials and all crocodiles have a pit-like structure, the follicle gland, near the posterior margin (figures 22 and 24). The glands are clearly visible in live specimens, raw skins, and crusts. In finished skins dyed dark, the follicle glands may be lighter in color (figure 25). The glands may be partly obscured during the polishing process. If this happens, the gland usually can be seen as a short deep wrinkle running from the gland to the posterior edge of the scale (figure 35). In finished skins, follicle glands are detected most easily on the throat and in the area just anterior to the vent as well as on the underside of the tail (figures 28 and 30).

SPIDER-WEB UMBILICUS. Alligator hides have neither surface pitting nor follicle glands. The ventral scales of finished alligator skins are glossy smooth without ornamentation. It is also possible to distinguish the belly skin of the American alligator (*Alligator mississippiensis*) from all other species on the basis of the shape of the umbilicus scar. Crocodilians with buttons in the ventral scales tend to lose all evidence of the umbilicus once it is healed and the osteoderms are formed. Other species may retain the posterior one-third of the scar as a zigzag arrangement of small scales scattered along the midline just anterior to the vent. In the American alligator, and no other species, this same

posterior portion of the scar remains as an area of soft skin lacking scales, and because of the profusion of creases and lines it has a distinctly spider-web appearance (figure 7).

VENTRAL COLLAR. Most crocodilians have a prominent row of enlarged scales, called a ventral collar, across the throat just anterior to the front legs (figure 1). A few species lack an enlarged row of scales, so the collar is not conspicuous. One species has a double collar, two enlarged rows of scales.

TRANSVERSE VENTRAL SCALE ROWS. Ventral scales are arranged in transverse rows in all crocodilians, but the number of rows found between the neck and vent differs between species. The transverse rows of ventral scales are counted from the first row posterior to the ventral collar to, but not including, the row of scales encircling the vent (figure 1). The row of scales around the vent may be missing from a commercial hide because the hide-hunter was careless when skinning the animal. In that event the position of the missing rows must be estimated. Only rows which cross the midline are counted. Incomplete or missing rows will add confusing variation to the count, so to eliminate doubt, the count first should be made only to the right of the midline and then repeated on the left side, and the two counts compared.

LARGE-SCALE AND SMALL-SCALE HIDES. Soft-belly crocodilians which have 26 to 35 transverse ventral scale rows are called small-scale hides by the hide trade. Soft-belly species with 20 to 25 transverse ventral scale rows are called large-scale hides (see the key that follows and figure 26).

TAIL WHORLS. The transverse rows of scales under the tail are the ventral portions of the whorls of scales that completely encircle the tail. The ventral portion of these whorls, like the rows of ventral scales on the body, are usually complete and evenly arranged (figure 28). Morelet's crocodile (*Crocodylus moreletii*), however, possesses irregular or incomplete whorls 66 percent of the time (figure 30). No other species shows as high an incidence of irregularity in this character.

IDENTIFICATION OF CROCODILIAN HIDES

The following keys can be used to identify the species, or species groups, of commercial belly skins, hornbacks, and sides. The keys are of limited use in identifying throats and girdles, and are useless for flippers. They may be useless in identifying skins already manufactured into finished products.

Keys are identification tools which employ a series of alternative choices. To use the keys, first decide whether or not the hide you wish to identify is a side, belly, or hornback. Once this determination has been made, proceed to the appropriate key. Each set of alternative choices, or couplets, is numbered. Starting with couplet 1, decide which of the two choices, "a" or "b," best describes the hide to be identified. The number that follows the correct choice indicates the next couplet. By moving from couplet to couplet following the numbers shown after each correct choice, you will arrive at a final choice which indicates the species, or species group, of crocodilian from which the hide was taken. Once the identification has been made, you should turn to the text that follows the keys for information on the distribution of the species, the commercial names under which it is sold, additional distinguishing characteristics, and status of the wild populations.

Species identifications supplied by manufacturers are not to be relied on until verified by means of the keys. In the past two years, the authors have seen live African slender-snouted crocodiles and South American caimans shipped into the United States from Bangkok, Thailand, as Siamese crocodiles; finished African dwarf crocodile hides enter from a tanner in France who labelled them gavial; and wallets made from South American caimans arrive from an Italian manufacturer who declared they were Nile crocodile.

A KEY TO COMMERCIAL CAIMAN SIDES

The key to sides is based on the assumption that the sides being identified are from caimans (*Caiman, Melanosuchus,* or *Paleosuchus*), and not from other species. At the present time, caimans are the only crocodilians being skinned in this manner. This may not be the case at some future date. In addition, small finished products such as belts may be pieced together from scrap left over from the manufacture of large belly hide products. These small pieces may come from any species, therefore, the key is of little use in identifying pieced items.

1. a) Rows of large oval scales alternating with rows of small scales (figure 21) . . . *Melanosuchus niger.*

    b) Rows of large scales alternating with network of creases and small irregular scales (figure 16) . . . 2

2. a) Large oval scales, usually smooth, and arranged in distinct rows . . . *Caiman crocodilus* (four subspecies), *Caiman latirostris.*

b) Large oval scales usually keeled, and usually not arranged in distinct rows ... *Paleosuchus palpebrosus, Paleosuchus trigonatus.*

A KEY TO CROCODILIAN BELLY SKINS AND HORNBACK SKINS

This key is for use with belly skins. Hornback hides can be identified if you limit your attention to the belly scales found along the lateral edges of the hide. Surface pitting is not evident in untanned hides.

1. a) Ventral (belly) scales with follicle glands (figures 22 and 24) ... 2
   b) Ventral (belly) scales without follicle glands (figures 4, 11, and 18) ... 4

2. a) Osteoderm buttons present (figures 34 through 37) ... *Crocodylus cataphractus, Crocodylus niloticus, Osteolaemus tetraspis* (two subspecies).
   b) Osteoderm buttons not present (figure 27) ... 3

3. a) Transverse rows of ventral scales 20 to 25 ... *Crocodylus acutus* (south of Panama), *Crocodylus intermedius, Crocodylus johnsoni, Crocodylus novaeguineae* (two subspecies), *Tomistoma schlegelii.*
   b) Transverse rows of ventral scales 26 to 35 ... *Crocodylus acutus* (north of Panama), *Crocodylus moreletii, Crocodylus niloticus, Crocodylus palustris* (two subspecies), *Crocodylus porosus, Crocodylus rhombifer, Crocodylus siamensis, Gavialis gangeticus.*

4. a) No osteoderm buttons present in midbelly (figure 6), or single buttons present (figure 8) ... 5
   b) Double osteoderm buttons present in midbelly (figures 14 and 19) ... 6

5. a) Umbilicus scar has spider-web appearance (figure 7); transverse rows of ventral scales 29 or more ... *Alligator mississippiensis.*
   b) Umbilicus scar not evident or lacks spider-web appearance; transverse rows of ventral scales 28 or fewer ... *Alligator sinensis.*

6. a) Large osteoderm buttons present medially only, not over pelvic girdle (figure 19); surface pitting slight; transverse rows of ventral scales 25 or more ... *Melanosuchus niger.*
   b) Large osteoderm buttons in all large ventral scales, throat to pelvis (figure

13); surface pitting slight to pronounced; transverse rows of ventral scales 18 to 30 ... 7

7. a) Surface pitting pronounced; transverse rows of ventral scales 20 to 30 ... 8
   b) Surface pitting slight or absent; transverse rows of ventral scales 18 to 22 ... *Paleosuchus palpebrosus, Paleosuchus trigonatus.*

8. a) Transverse rows of ventral scales 26 to 30; double ventral collar ... *Caiman latirostris.*
   b) Transverse rows of ventral scales 20 to 27; single ventral collar ... *Caiman crocodilus* (four subspecies).

In the text that follows, the species and subspecies are listed alphabetically by scientific name within each family. The systematic arrangement follows Wermuth and Mertens (1961).

Family ALLIGATORIDAE

AMERICAN ALLIGATOR

Alligator mississippiensis (Daudin)

DISTRIBUTION. Southeastern United States — the states of North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, and Texas. This species does not occur outside the United States (Schmidt, 1953; U.S. Department of Interior, 1968; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called the Florida and the Mississippi alligator, or gator. Hides are marketed as American, Florida, or Louisiana alligator or soft-belly.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. No osteoderm buttons (large specimens from Florida have single osteoderm buttons on the throat). Transverse ventral scale rows 29 or more. Umbilicus scar prominent and with spider-web appearance. Maximum length of live specimen is 18 feet.

STATUS OF WILD POPULATIONS. Endangered (Honegger, 1968; Pan American Union, 1967; U.S. Department of Interior, 1968). Now protected by state law in every state in which it occurs; by federal prohibition on interstate traffic in illegal hides; and by local and state prohibitions on sales of live specimens, hides, and hide products.

CHINESE ALLIGATOR

Alligator sinensis Fauvel

DISTRIBUTION. The lower Yangtze River drainage of China (Pope, 1935; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In China it is called *T'o, Tou Lung, Yow Lung.*

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Usually no osteoderm buttons, but occasionally single buttons may be present in the midbelly and collar areas. Transverse ventral scale rows 28 or fewer. Umbilicus scar not evident. Maximum length of live specimen is 6½ feet.

STATUS OF WILD POPULATIONS. Probably endangered (Honegger, 1968). This species is known from a strip of territory only a few hundred miles long. A. F. Oeming of the Alberta Game Farm, Canada, recently returned from a trip to China and reported (in *litt.*) that the species is totally protected by law and the law is rigidly enforced. Dr. Cheng, of the Institute of Zoology, Academia Sinica, Peking, is studying the species.

SOUTH AMERICAN CAIMAN

Caiman crocodilus crocodilus (Linnaeus)

DISTRIBUTION. Northern South America — Colombia east of the Andes, Peru, Ecuador, Venezuela, Guyana, Surinam, French Guiana, Trinidad, and, with the exception of a few southern tributaries, the Amazon drainage of Brazil [the exceptions are listed under *Caiman crocodilus yacare*] (Carvalho, 1955; Medem, 1968; Schmidt, 1928b; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In the United States it is frequently called spectacled caiman (and equally frequently given the old synonymous scientific name *Caiman sclerops*). In Central and South America it is called "alligator," *baba, babilla, cachirré, caimán, caimán blanco, caimán del Paraguay, cascarudo, cocodrillo, jacaré, jacaré de Lunetas, jacaretinga, lagarto, lagarto blanco, lagarto negro, ocoroche, tinga,* and *yacaré blanco.* Hides are frequently marketed under these names.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons present. Surface pitting evident. Transverse ventral scale rows 20 to 24. Single prominent ventral collar. Highest point of ventral scale slightly anterior of center (figure 39). *Sides:* Rows of large oval scales alternating with network of small irregular scales and creases. (The network is actually soft skin folds and creases without scales.) Maximum length of live specimen is 8½ feet.

STATUS OF WILD POPULATIONS. Most wild populations are declining and some have all but disappeared due to slaughter by hide hunters and capture by live animal collectors (Pan American Union, 1967). The subspecies is considered endangered by some experts (Honegger, 1968). South American countries require that hides be tanned before export. Colombia protects specimens less than 1.2 meters in length (Medem, 1970, in *litt.*) and Peru protects those less than one meter long (Crowe, 1965).

RIO APAPORIS CAIMAN

Caiman crocodilus apaporiensis Medem

DISTRIBUTION. Colombia — known only from the Apaporis River and its tributaries between the Falls of Jirijirimo and Puerto Yaviya (Medem, 1955, 1968; Wermuth and Mertens, 1961).

OTHER COMMON NAMES. In Colombia it is called *babilla, cachirré, cocodrillo, jacaretinga, lagarto negro* and *ocoroche.* If marketed, hides would be sold under these names, or as *tinga.*

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons present. Surface pitting evident. Transverse ventral scale rows 20 to 24. Single prominent ventral collar. Highest point of ventral scale is slightly anterior of center (figure 39). *Sides:* Rows of large oval scales alternating with network of small irregular scales and creases. Maximum length of live specimen is 7 feet.

STATUS OF WILD POPULATIONS. Critically endangered. This subspecies has the most restricted range of any crocodilian. It is known only from an area 125 miles long in one river. Hide hunters can completely decimate this form in one or two years unless hunting is prohibited immediately. Colombia prohibits the export of untanned hides and protects specimens less than 1.2 meters in length (Medem, 1970, in *litt.*).

BROWN CAIMAN

Caiman crocodilus fuscus (Cope)

DISTRIBUTION. Central America — southern Mexico to Colombia, west of the Andes (Medem, 1968; Schmidt, 1928b; Smith and Taylor, 1950; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In the United States it is also called spectacled caiman, Central

American caiman, dusky caiman, and Magdalena caiman. In Central America it is called "alligator," *caimán, cocodrillo,* and *cuajipal.* In Colombia it is known as *babilla, lagarto negro,* and *jacaretinga.* Hides are marketed under these names, and Central American *tinga.*

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons present. Surface pitting evident. Transverse ventral scale rows 20 to 24. Single prominent ventral collar. Highest point of ventral scale slightly anterior of center (figure 39). *Sides:* Rows of large oval scales alternating with network of small irregular scales and creases. Maximum length of live specimen is 7 feet.

STATUS OF WILD POPULATIONS. Many wild populations are disappearing due to hide-hunting (Pan American Union, 1967). The subspecies is considered endangered by some experts (Honegger, 1968). South American countries prohibit the export of untanned hides. Colombia and Panama protect specimens less than 1.2 meters in length (Medem, 1970, in *litt;* D. Tovar, 1970, in *litt.*). Peru protects specimens less than 1.5 meters in length (Honegger, 1968). Mexico's laws regulate hunting of this species.

YACARE

Caiman crocodilus yacare (Daudin)

DISTRIBUTION. Southern South America — specifically the Paraguay and Parana river drainage systems of Paraguay, Uruguay, Argentina, and Brazil, and the southern tributaries of the Amazon in Bolivia [the Mamore, Itenez, and Beni drainages] and Brazil [the Guapore drainage, and the Araguaia River above its confluence with the Tapirape] (Carvalho, 1955; Medem, 1968; Schmidt, 1928b; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called the Paraguay caiman and red caiman in the United States. In South America it is called *caimán del Paraguay, cascarudo, jacaré, jacaré de Lunetas, jacaretinga, lagarto, tinga, yacaré,* and *yacaré de hocico angosto.* Hides are marketed under these names.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons present. Surface pitting evident. Transverse ventral scale rows 20 to 25. Single prominent ventral collar. Highest point of ventral scale is slightly anterior of center (figure 39). *Sides:* Rows of large oval scales alternating with network of ir-

regular shaped small scales and creases. Maximum length of live specimen is 8 feet.

STATUS OF WILD POPULATIONS. Endangered (Pan American Union, 1967; U.S. Department of Interior, 1970). Most wild populations declining in numbers (Jose Cei, 1970, in *litt.*). South American countries prohibit the export of untanned hides. Its import is prohibited under provisions of the Endangered Species Conservation Act (U.S. Department of Interior 1970).

BROAD-SNOUTED CAIMAN

Caiman latirostris (Daudin)

DISTRIBUTION. Southern South America — the drainages of the Paraguay, Parana, and Uruguay rivers in Argentina, Uruguay, Paraguay, and Brazil, and the rivers emptying into the southeast coast of Brazil south of Recife (Carvalho, 1955; Medem, 1968; Schmidt, 1928b; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In South America it is called *jacaré de Papo Amarelo, overo, ururau,* and *yacaré de hocico ancho.* Hides are marketed under these names.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons present. Surface pitting slight or absent. Transverse ventral scale rows 26 to 30. Double ventral collar. Highest point of ventral scale located at center of scale. *Sides:* Rows of large oval scales alternating with network of irregular shaped small scales and creases. Maximum length of live specimen is 9 feet.

STATUS OF WILD POPULATIONS. Endangered (Pan American Union, 1967). This species is nearly extinct from excessive hide hunting (Jose Cei, 1970, in *litt.*). South American countries prohibit export of untanned hides.

BLACK CAIMAN

Melanosuchus niger (Spix)

DISTRIBUTION. Northern and central South America — Amazon basin drainages of Brazil, Colombia, Venezuela, Guyana, Peru, and Bolivia (Carvalho, 1955; Medem, 1963, 1968; Schmidt, 1928b; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In South America it is called *asu, caimán, caimán negro, cocodrillo, jacaré açú, jacaré assú, jacaré asú, jacaré uassú, jacaré una,* and *yacaré assú.* Hides are marketed under these names.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons, at least medially. Lateral scales may lack osteoderms or possess small osteoderms in center of scales. Surface pitting slight. Transverse ventral scale rows 25 to 28. *Sides:* Parallel rows of large oval scales alternating with rows of small oval scales. Maximum length of live specimen is 16 feet.

STATUS OF WILD POPULATIONS. Endangered (Honegger, 1968; Pan American Union, 1967). Rapidly declining everywhere, and exterminated in many areas. South American countries prohibit the export of untanned hides. Peru prohibits the killing of specimens less than 2 meters in length (Honegger, 1968).

DWARF CAIMAN
Paleosuchus palpebrosus (Cuvier)

DISTRIBUTION. Northern and central South America — Amazon and Orinoco river drainages of Colombia, Venezuela, Guyana, Brazil, Peru, Ecuador, and Bolivia (Carvalho, 1955; Medem, 1967, 1968; Schmidt, 1928b; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called musky caiman and Cuvier's smooth-fronted caiman. In South America it is called *cachirré, jacaré coroá,* and *yacaré coroá.* Hides are marketed under these names.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons on all large ventral scales. Surface pitting slight or absent. Transverse ventral scale rows 18 to 22. Single prominent ventral collar. *Sides:* Large scales scattered, not in well-defined rows, and separated by wide areas of soft skin. Maximum length of live specimen is 5½ feet.

STATUS OF WILD POPULATIONS. Declining in numbers (Pan American Union, 1967). *Paleosuchus* is possibly the least persecuted of the crocodilians at the present time. Its small size and heavy osteoderm buttons make the skins less desirable than skins from the larger caimans and crocodiles of South America. South American countries prohibit the export of untanned hides.

SMOOTH-FRONTED CAIMAN
Paleosuchus trigonatus (Schneider)

DISTRIBUTION. Northern and central South America — the Amazon and Orinoco river drainages of Colombia, Venezuela, Guyana,

Brazil, Ecuador, Peru, and Bolivia (Carvalho, 1955; Medem, 1967, 1968; Schmidt, 1928b; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called Schneider's smooth-fronted caiman. In South America it is called *cachirré, jacaré coroá, jacaré curuá,* and *yacaré coroá.* Hides are marketed under these names.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* No follicle glands. Full double osteoderm buttons on all large ventral scales. Surface pitting slight or absent. Transverse ventral scale rows 18 to 22. Single prominent ventral collar. *Sides:* Scattered large keeled oval scales, not in well-defined rows, and separated by wide areas of soft skin. Maximum length of live specimen is 7 feet.

STATUS OF WILD POPULATIONS. Declining in numbers (Pan American Union, 1967). *Paleosuchus* is possibly the least persecuted of the crocodilians. Its small size and heavy ossification of the osteoderms makes the skins less desirable than skins from the larger caimans and crocodiles of South America. South American countries prohibit the export of untanned hides.

Family CROCODYLIDAE

AMERICAN CROCODILE
Crocodylus acutus Cuvier

DISTRIBUTION. Florida, West Indies, Central and northern South America — southern Florida, Cuba, Hispaniola (Haiti and Dominican Republic), Jamaica, Mexico south to Colombia and Venezuela, exclusive of the Orinoco river drainage system (Cochran, 1941; Medem, 1968; Smith and Taylor, 1950; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In Central America and Cuba it is called *caimán,* and in South America it is known as *caimán* and *caimán de aguja.* Hides may be marketed under these names, or simply as Central or South American "alligator," crocodile, soft-belly, small scale (north of Panama) or large scale (south of Panama).

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 25 to 35. Tail whorls regular ventrally. Maximum length of live specimen is 23 feet.

STATUS OF WILD POPULATIONS. Declining everywhere due to excessive hide-hunting (Pan American Union, 1967). The species is considered endangered by some experts (Honegger, 1968). Many populations in Central and South America have been totally exterminated. The species is protected by state law in Florida, and South American countries prohibit the export of untanned hides. Mexico regulates the hunting of the species, as does Nicaragua. Jamaica prohibits the export of crocodiles, their eggs, or skins (K.C. Hall, 1970, in *litt.*). The species is protected in Cuba and Colombia, although the law is not enforced in the latter (Honegger, 1968).

AFRICAN SLENDER-SNOUTED CROCODILE

Crocodylus cataphractus Cuvier

DISTRIBUTION. Western and central Africa — the Congo, Niger, and Volta river drainages, and the coastal rivers from Senegal south to northern Angola. Only once recorded from East Africa at Ujiji, Tanzania, on Lake Tanganyika (Schmidt, 1919; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is sometimes called the West African crocodile, African long-nosed crocodile, African gavial, or sub-water crocodile. Hides are sold under these names or as Nigerian, Congo, or Cabinde "alligator," crocodile, or button hides.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. Round or elliptical single osteoderm buttons present. Surface pitting may or may not be present. Transverse ventral scale rows 25 to 29. Hides from Nigeria usually are missing the tip of the tail, due to local hunting practices. Skins from other parts of Africa usually have complete tails. Maximum length of live specimen is 13 feet.

STATUS OF WILD POPULATIONS. Critically endangered (A. C. Pooley, 1971, personal communication). This species is limited to large rivers, and is rarely abundant anywhere. Populations are declining everywhere due to hide hunting and the spread of human population (Lowes, 1970).

ORINOCO CROCODILE

Crocodylus intermedius Graves

DISTRIBUTION. Northern South America — the Orinoco river drainage of Colombia (east of the Andes), Venezuela, and possibly Guyana (Medem, 1968; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is called *caimán* in South America. It is marketed under this name, or as Colombian, Venezuelan, or Venezuelan delta "alligator," crocodile, large scale, or soft-belly.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 20 to 25. Tail whorls usually regular. Maximum length of live specimen is 23 feet.

STATUS OF WILD POPULATIONS. Endangered (Pan American Union, 1967; U.S. Department of Interior, 1970). Because of excessive hide hunting the species is now rare in Venezuela, and apparently exterminated in Colombia (Honegger, 1968). South American countries prohibit the export of untanned hides. Colombia has legislation prohibiting the hunting of crocodiles, but it is not enforced (Honegger, 1968). Import is prohibited under provision of the Endangered Species Conservation Act (U.S. Department of Interior, 1970).

JOHNSON'S CROCODILE

Crocodylus johnsoni Krefft

DISTRIBUTION. Northern Australia — from the Fitzroy River in northern Western Australia to Mackay in eastern Queensland (Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933; Worrell, 1963).

OTHER COMMON NAMES. In Australia it is called the freshwater crocodile, Johnson's river crocodile, Johnstone's crocodile, and fish crocodile. It may be marketed under these names, or as Australian or Singapore "alligator," gator, crocodile, soft-belly, or large scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 22 to 24. Tail whorls usually regular. Maximum length of live specimen is 9½ feet.

STATUS OF WILD POPULATIONS. Rare (Honegger, 1968). The species is completely protected by law in Western Australia and Northern Territories, but skins are still shipped from Queensland (Fauna Preservation Society, 1970b; Green, 1969; Honegger, 1968).

MORELET'S CROCODILE

Crocodylus moreletii Duméril, Bibron and Duméril

DISTRIBUTION. Northern Central America — Atlantic and Pacific coasts of Mexico, British Honduras, and Guatemala (Smith and Taylor,

1950; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is sometimes called Belize crocodile or Central American crocodile. In Central America it is called "alligator," *caimán*, and *lagarto de El Petén*. Hides are marketed under these names, or as Mexican "alligator," crocodile, small scale, or soft-belly.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 27 to 32. Tail whorls irregular (66 percent of the time). Maximum length is 8 feet.

STATUS OF WILD POPULATIONS. Endangered (Honegger, 1968; Pan American Union, 1967; U.S. Department of Interior, 1970). This species has all but been eliminated from British Honduras and parts of Guatemala (Charnock-Wilson, 1970). It is still locally abundant in parts of Mexico (Fauna Preservation Society, 1969b). Mexico has protective laws but they are unenforced (Honegger, 1968). Guatemala began enforcing its protective legislation in 1970. Importation is prohibited under provision of the Endangered Species Conservation Act (U.S. Department of Interior, 1970).

NILE CROCODILE
Crocodylus niloticus Laurenti

DISTRIBUTION. Africa (all of Africa except the northwest corner and central Sahara); east along the Mediterranean coast to Syria; Malagasy Republic (Madagascar); and Seychelles, Comoros, and Mauritius (Schmidt, 1919; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called the Nilotic crocodile. Hides are marketed as African, Ethiopian, Kenya, Madagascan, or Nile "alligator," "caiman," crocodile, small scale, button-belly, or soft-belly.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. Usually no buttons, but occasionally single buttons may be present in the midbelly and collar area. Transverse ventral scale rows 26 to 32. Tail whorls usually regular. Hides from Nigeria have the tip of the tail missing due to local hunting practices. The tails are complete on hides from elsewhere. Maximum length of live specimen is probably 18 feet.

STATUS OF WILD POPULATIONS. Endangered (Cott, 1961; Honegger, 1968; Pooley,

1969; U.S. Department of Interior, 1970). This species has been exterminated over large areas of Africa by hide hunters (Fauna Preservation Society, 1969c, 1969d, 1970a; Lowes, 1970; Pooley, 1970, in *litt.*). It can be found in numbers only in small local populations. It is extinct in the Seychelles and Mauritius. It is protected by law in most East African countries and in national parks and game preserves (Cott, 1969). Hunting of this species is to be regulated throughout all of Africa by the African Convention for the Conservation of Nature and Natural Resources (Burhenne, 1970; Honegger, 1968). South Africa has set up a research program in hopes of saving the species and restocking it in areas where it has been exterminated (Pooley, 1970). Importation is prohibited under provision of the Endangered Species Conservation Act (U.S. Department of Interior, 1970).

NEW GUINEA CROCODILE
Crocodylus novaeguineae novaeguineae Schmidt

DISTRIBUTION. New Guinea (Schmidt, 1928a, 1932; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called the New Guinea freshwater crocodile. Hides may be marketed as Australia, New Guinea, or Singapore "alligator," crocodile, soft-belly, or large scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 24 to 25. Tail whorls usually regular. Maximum length of live specimen is 9½ feet.

STATUS OF WILD POPULATIONS. Rare (Honegger, 1968). Populations are declining rapidly due to hide hunting. Specimens over 20 inches in belly width are protected by laws in most of Papua and Northeast New Guinea (Bustard, 1970; Fauna Preservation Society, 1969a; Honegger, 1968).

PHILIPPINE CROCODILE
Crocodylus novaeguineae mindorensis Schmidt

DISTRIBUTION. Philippine Islands—Luzon, Mindoro, and Mindanao Islands (Schmidt, 1935; Wermuth, 1953; Wermuth and Mertens, 1961).

OTHER COMMON NAMES. Also called the Mindoro crocodile and Philippine freshwater crocodile. Hides may be marketed under the name Philippine or Singapore "alligator," crocodile, soft-belly, or large scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 24 to 26. Tail whorls usually regular. Maximum length of live specimen is 8 feet.

STATUS OF WILD POPULATIONS. Rare, possibly endangered. Hide hunting is eliminating the species from parts of its former range.

MUGGER CROCODILE
Crocodylus palustris palustris Lesson

DISTRIBUTION. India and Pakistan — from the Dasht River in West Pakistan through all the river systems of India to the Brahmaputra River drainage in the east (De Rooij, 1915; Schmidt, 1935; Smith, 1931; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. Also called the marsh crocodile, broad-snouted crocodile, swamp crocodile, and Indian freshwater crocodile. Hides may be marketed as Indian "alligator," crocodile, soft-belly, or small scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 26 to 32. Ventral collar not distinct (no enlarged scales). Tail whorls usually regular. Maximum length of live specimen is 13 feet.

STATUS OF WILD POPULATIONS. Endangered. The species is protected in India by a ban on the export of crocodile hides, and in Pakistan by a ban on the export of all wild animal hides (Fauna Preservation Society, 1967, 1970c; Mountfort, 1969).

CEYLON MUGGER CROCODILE
Crocodylus palustris kimbula Deraniyagala

DISTRIBUTION. Ceylon (Deraniyagala, 1936, 1939, 1953; Wermuth, 1953; Wermuth and Mertens, 1961).

OTHER COMMON NAMES. It is also called the Ceylon swamp crocodile, Ceylon marsh crocodile, and lake crocodile. In Ceylon it is known as *hale kimbula, ala kimbula,* and *kulathi muthele.* It may be marketed as Ceylon "alligator," crocodile, soft-belly, or small scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 26 to 32. Ventral collar present and distinct. Tail whorls regular. Maximum length of live specimen is 18 feet.

STATUS OF WILD POPULATIONS. Declining in numbers. Hunting is regulated by the Ceylon government (Fauna Preservation Society, 1970e).

SALTWATER CROCODILE
Crocodylus porosus Schneider

DISTRIBUTION. India and Ceylon east to Australia and New Guinea — the coastal rivers, lagoons, and marshes from Cochin in extreme southwestern India east to Ceylon, Burma, Malaysia, Thailand, Cambodia, Vietnam, Indonesia, the Philippines, Palau Islands, northern Australia, New Guinea, Solomon Islands, New Hebrides, and Fiji (Deraniyagala, 1939, 1953; De Rooij, 1915; Schmidt, 1932; Taylor, 1970; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933; Worrell, 1963).

OTHER COMMON NAMES. It is also called the estuarine crocodile, gator (in Australia), and sea-going crocodile. In Ceylon it is known as *pita gatteya, gatte kimbula, gorekeya,* and *semmukhan;* in Indonesia, *buaja;* in Malaysia, *buaja, buaya, baya,* and *rawing.* Hides may be marketed under these names, or as Indian, Javan, Philippine, Singapore, Sumatran, or Thailand "alligator," crocodile, soft-belly, or small scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 30 to 35. Tail whorls regular. Maximum length of live specimen is probably 25 feet.

STATUS OF WILD POPULATIONS. Most populations are declining rapidly due to hide hunting, and the species is non-existent in some parts of its former range where it was once abundant (Fauna Preservation Society, 1970d; Honegger, 1968). It is partially protected in most of Papua and North East New Guinea, where specimens over 20 inches belly width may not be killed (Fauna Preservation Society, 1969a; Bustard, 1970). The species is completely protected in Western Australia until 1980 (Fauna Preservation Society, 1970b; Honegger, 1968). Indonesia has imposed size limits. Ceylon, India, and Pakistan protect the species completely by banning the export of all crocodile skins or the skins of all wild animals (Fauna Preservation Society, 1967; Honegger, 1968; Mountfort, 1969). Singapore requires export licenses. Deraniyagala (1939, 1953) mistakenly listed this species as occurring on the Seychelles and Mauritius where *Crocodylus niloticus* was known to occur in the past.

CUBAN CROCODILE
Crocodylus rhombifer Cuvier

DISTRIBUTION. Cuba and the Isle of Pines (Barbour and Ramsden, 1919; Varona, 1966; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In Cuba it is called *cocodrilo, cocodrilo perla, cocodrilo criollo, cocodrilo legitimo, caimán,* and occasionally *zaquendo.*

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 32 to 33. Tail whorls regular. Maximum length of live specimen is 16 feet.

STATUS OF WILD POPULATIONS. Endangered (Honegger, 1968; U.S. Department of Interior, 1970). The species once occurred on the Isle of Pines from which it has been exterminated. Today it only occurs in remnants of the Zapata Swamp on the south coast of Cuba, but hide hunting and land drainage has made it very nearly extinct even there. The Cuban government protects this species rigidly and has established a captive breeding facility in the Zapata Peninsula National Park in an attempt to save it from extinction (Honegger, 1968). Importation is prohibited under provision of the Endangered Species Conservation Act (U.S. Department of Interior, 1970).

SIAMESE CROCODILE
Crocodylus siamensis Schneider

DISTRIBUTION. Southeast Asia—Thailand, Cambodia, Vietnam, and Java (De Rooij, 1915; Smith, 1931; Taylor, 1970; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It may also be called the Siamese freshwater crocodile. In Indonesia it is called *buaja.* Hides may be sold as Java, Singapore, or Thailand "alligator," crocodile, soft-belly or small scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 30 to 34. Tail whorls regular. Maximum length of live specimen is 13 feet.

STATUS OF WILD POPULATIONS. Endangered. It has always been a rare animal in Indonesia, and became scarce in Thailand 30 years ago due to hide hunting. Today fewer than 200 remain in the wild in Thailand, but approximately 9,000 specimens are protected in the Sumatprakan Crocodile Farm in Bangkok (U.

Youngparpakorn, 1971, personal communication).

WEST AFRICAN DWARF CROCODILE
Osteolaemus tetraspis tetraspis Cope

DISTRIBUTION. West Africa — the Niger and Senegal river drainages and other rivers south of the Sahara and north of the Congo River drainage (Schmidt, 1919; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called the broad-snouted crocodile. Hides may be marketed as African "caiman," button-belly, bony crocodile, black crocodile, or rough-back crocodile.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. Large single osteoderm buttons present. Surface pitting usually evident. Transverse ventral scale rows 21 to 27. Maximum length of live specimen is 6½ feet.

STATUS OF WILD POPULATIONS. Endangered (A. C. Pooley, 1971, personal communication). Populations declining due to hide hunting, destruction of habitat, and live animal collecting (Lowes, 1970). This species has never been as abundant as the other African species.

CONGO DWARF CROCODILE
Osteolaemus tetraspis osborni (Schmidt)

DISTRIBUTION. Central Africa—the Congo River drainage (Schmidt, 1919; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. Also called the Central African dwarf crocodile, Osborn's dwarf crocodile, and African broad-snouted crocodile. Hides may be marketed as African "caiman," button-belly, bony crocodile, black crocodile, or rough-back crocodile.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. Large single osteoderm buttons present. Surface pitting usually evident. Transverse ventral scale rows 21 to 27. Maximum length of live specimen is 5 feet.

STATUS OF WILD POPULATIONS. Endangered (A. C. Pooley, 1971, personal communication). This species does not occur in large populations. Its numbers are declining due to hide hunting.

FALSE GAVIAL
Tomistoma schlegelii (Muller)

DISTRIBUTION. Southeast Asia — Indonesia (Kalimantan and Sumatra) and Malaysia

(De Rooij, 1915; Taylor, 1970; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. It is also called the Malay gavial, Malayan gharial, and Malayan fish crocodile. In Indonesia it is called *bediai sampit* and *buaja sapit;* in Malaya, *buaya senjulong;* in Sarawak, *baya kanulong.* Hides may be sold under these names, or as Singapore "alligator," crocodile, soft-belly, or large scale.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 22 to 24. Tail whorls usually regular. Maximum length of live specimen is 16 feet.

STATUS OF WILD POPULATIONS. Declining in numbers, soon to be endangered. Hide hunters have so decimated the populations of this animal in Malaysia that protective legislation is being considered (Lucas Chin, 1970, personal communication).

Family GAVIALIDAE

GAVIAL

Gavialis gangeticus (Gmelin)

DISTRIBUTION. India, Pakistan, and Burma — specifically the Indus, Mahandi, Ganges, Brahmaputra, and Kaladan river drainage systems, and possibly parts of the Irawaddy system in northwestern Burma (Smith, 1931; Wermuth, 1953; Wermuth and Mertens, 1961; Werner, 1933).

OTHER COMMON NAMES. In India it is called *gharial.* Hides may be sold as Indian soft-belly, small scale, "alligator," "crocodile," or gavial.

DISTINGUISHING CHARACTERISTICS OF COMMERCIAL HIDES. *Belly skins:* Follicle glands present. No osteoderm buttons. Transverse ventral scale rows 30 to 31. Ventral collar not prominent. Maximum length of live specimen is 21½ feet.

STATUS OF WILD POPULATIONS. Endangered (U.S. Department of Interior, 1970). Protected in India by a ban on the export of all crocodilian hides, and in Pakistan by a ban on the export of all wild animal hides (Fauna Preservation Society, 1967, 1970c; Mountfort, 1969). Importation is prohibited under provisions of the Endangered Species Conservation Act (U.S. Department of Interior, 1970).

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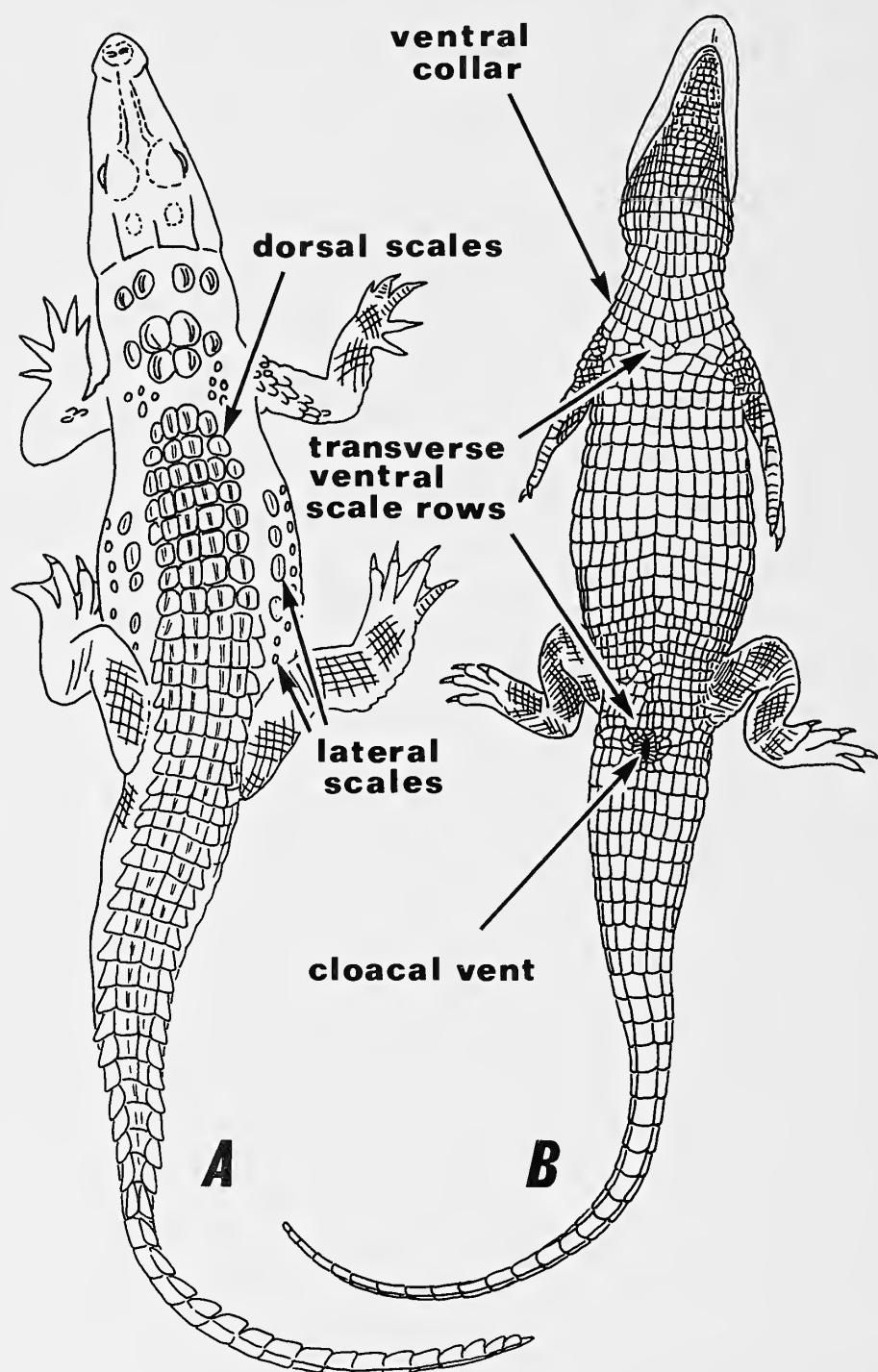
FIGURE 1. Diagrammatic dorsal (A) and ventral (B) views of a crocodilian. Hornback hides consist of most of the skin seen in A (skull and feet are absent). Belly hides consist of most of the skin seen in B (skull and feet are absent and the lateral [side] skin is attached). Transverse scale rows are counted by beginning and ending with the rows indicated by the arrows.
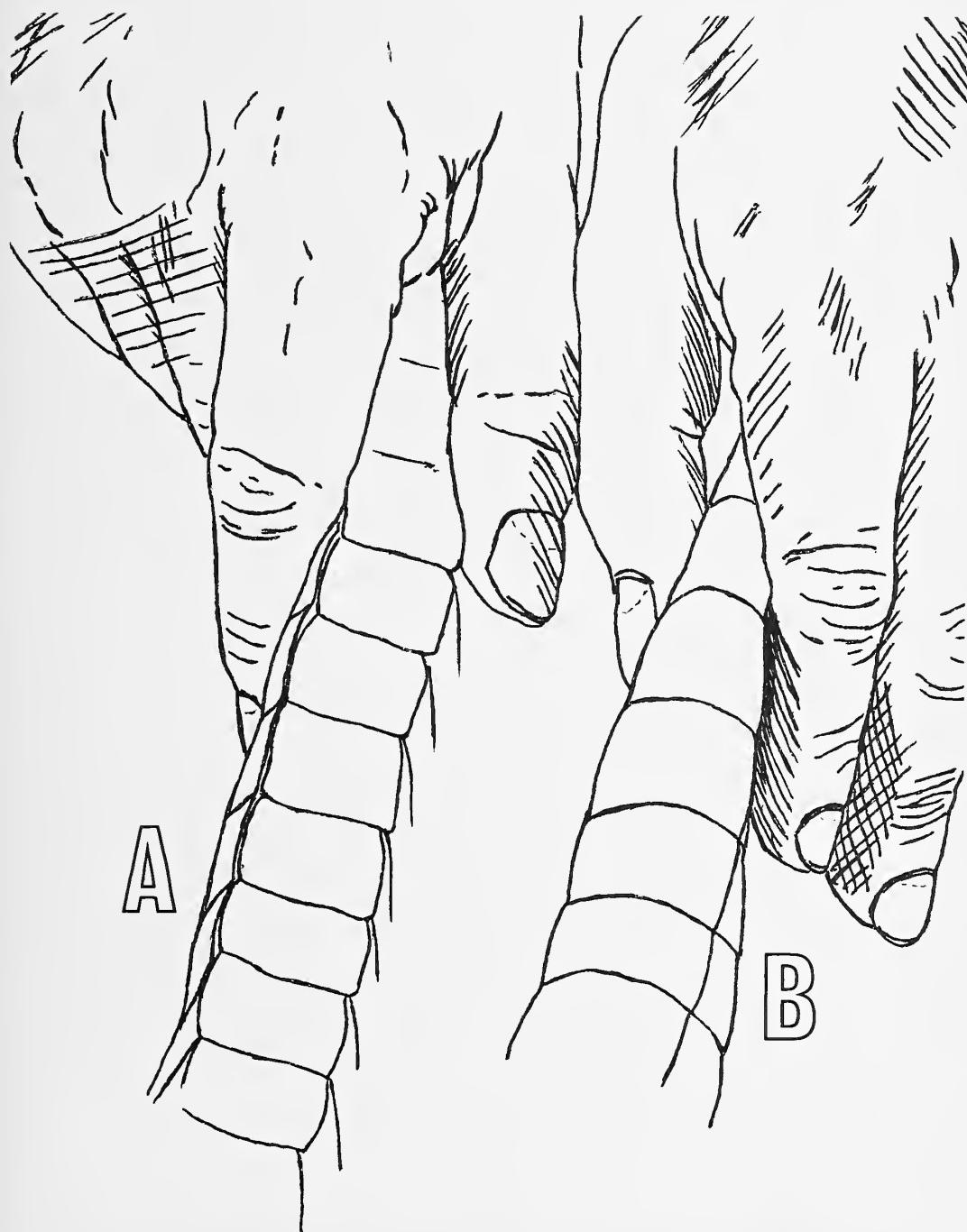
FIGURE 2. Comparative flexibility of a button-belly (A) and a soft-belly (B) hide. The hard osteoderms in the scales permit the button-belly hide to flex only between the scales, while the soft-belly hide will also flex through the scales.
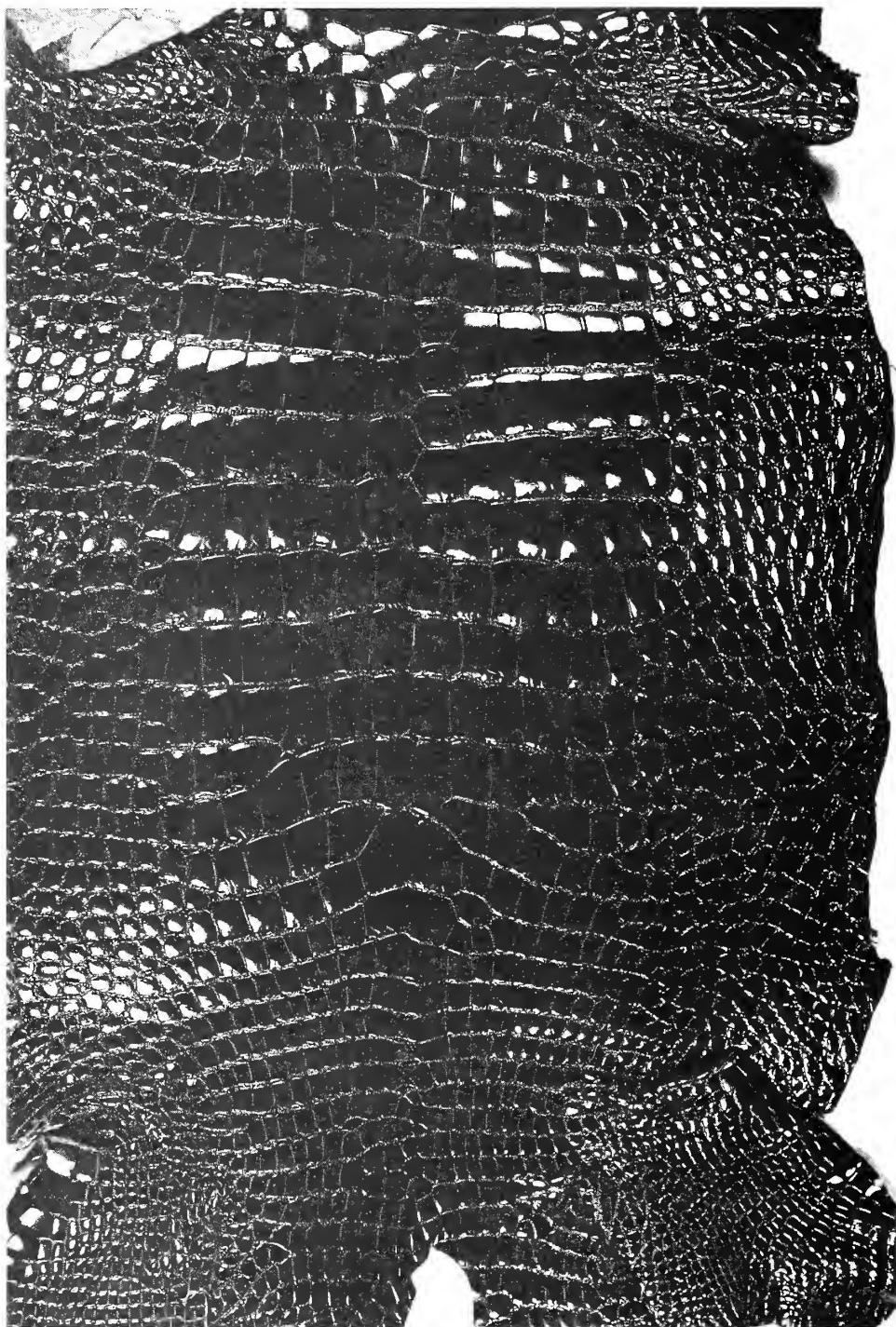
FIGURE 3. Outside surface of a finished American alligator (*Alligator mississippiensis*) belly hide. Closer views of the ventral scales and spider-web umbilicus are provided in figures 5 and 7.
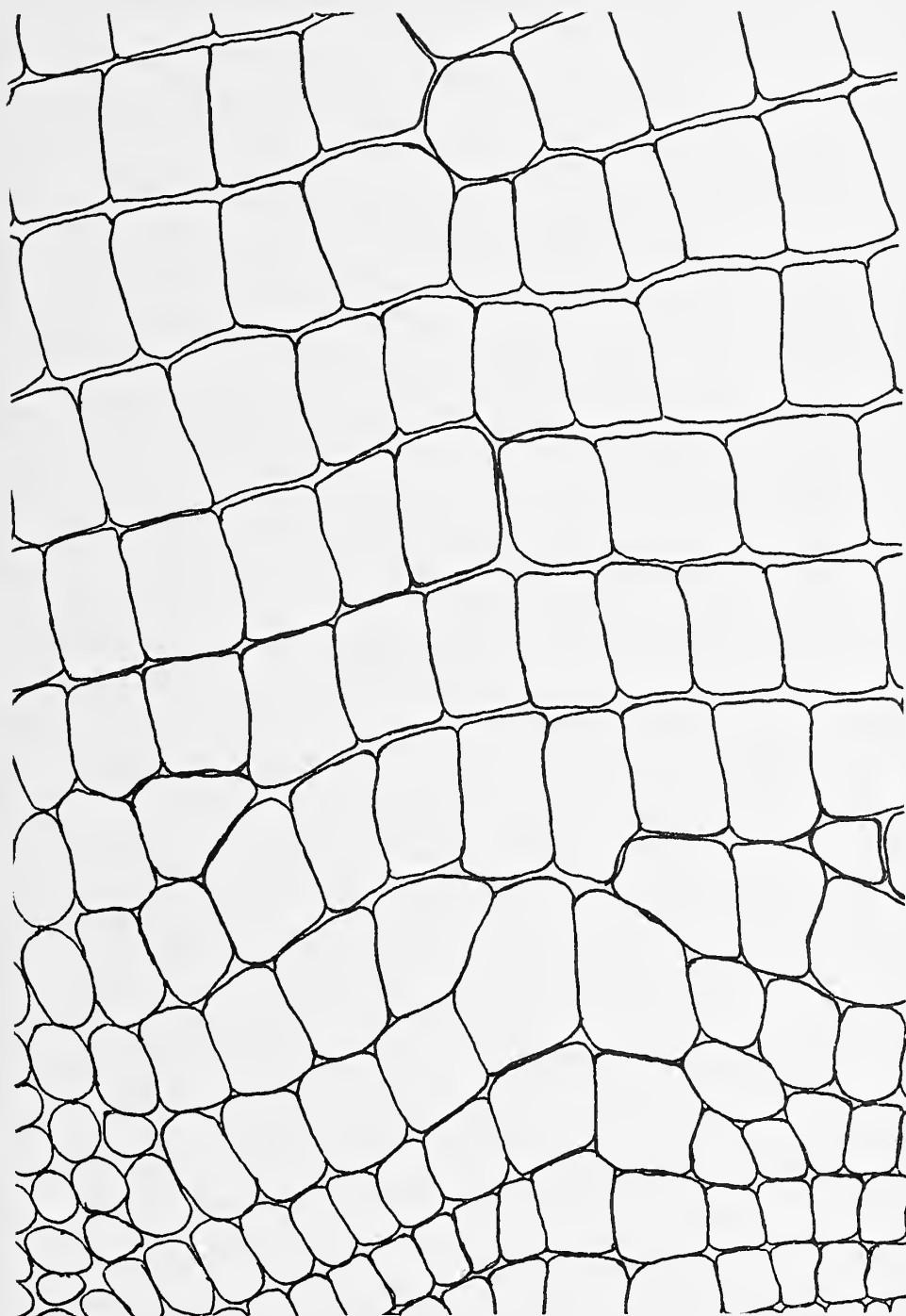
FIGURE 4. Diagrammatic illustration of the American alligator ventral scales shown in figure 5. Note the lack of both surface pitting and follicle glands.
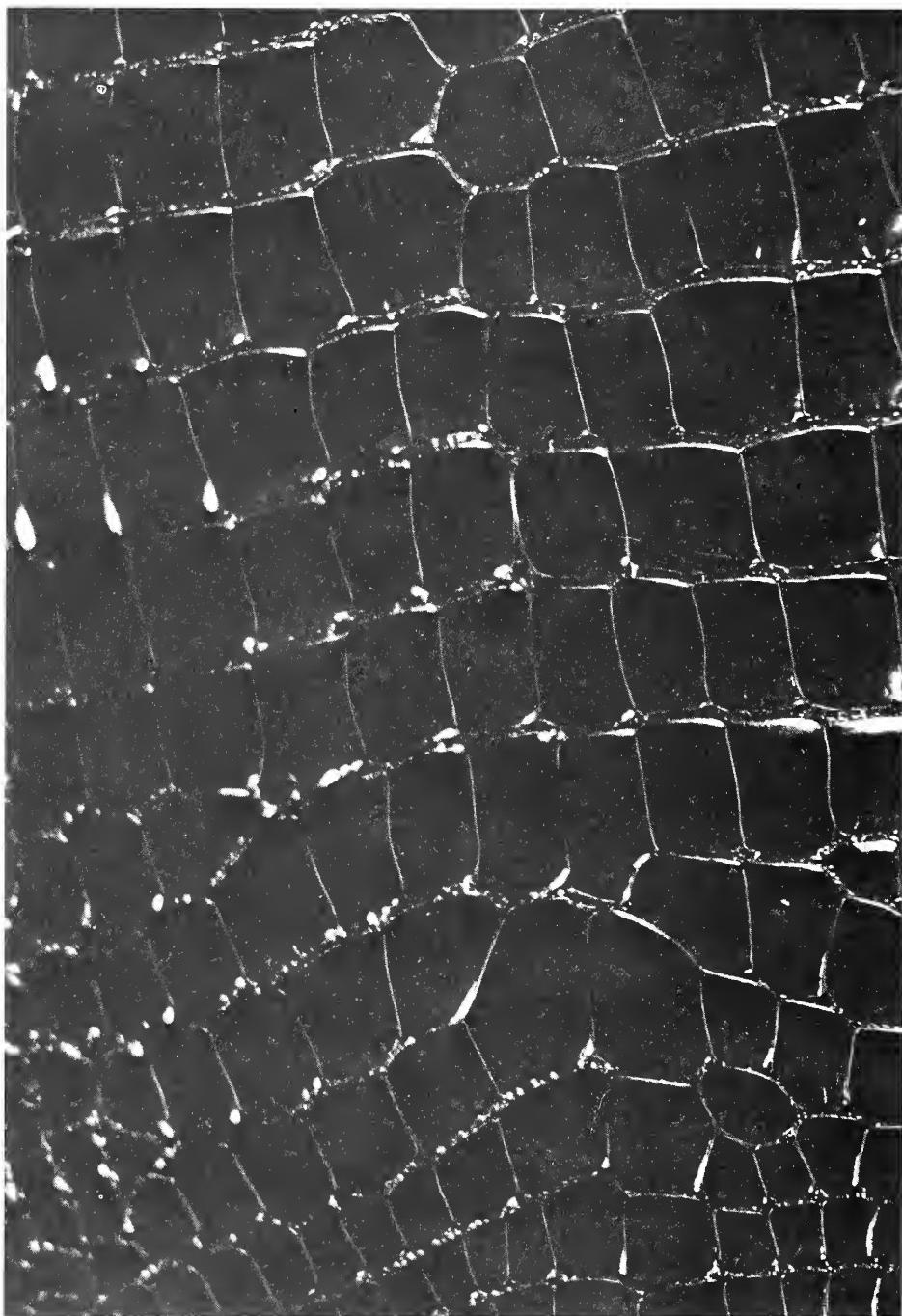
FIGURE 5.   Ventral scales of a finished American alligator (*Alligator mississippiensis*) belly hide. Compare it with figure 4.
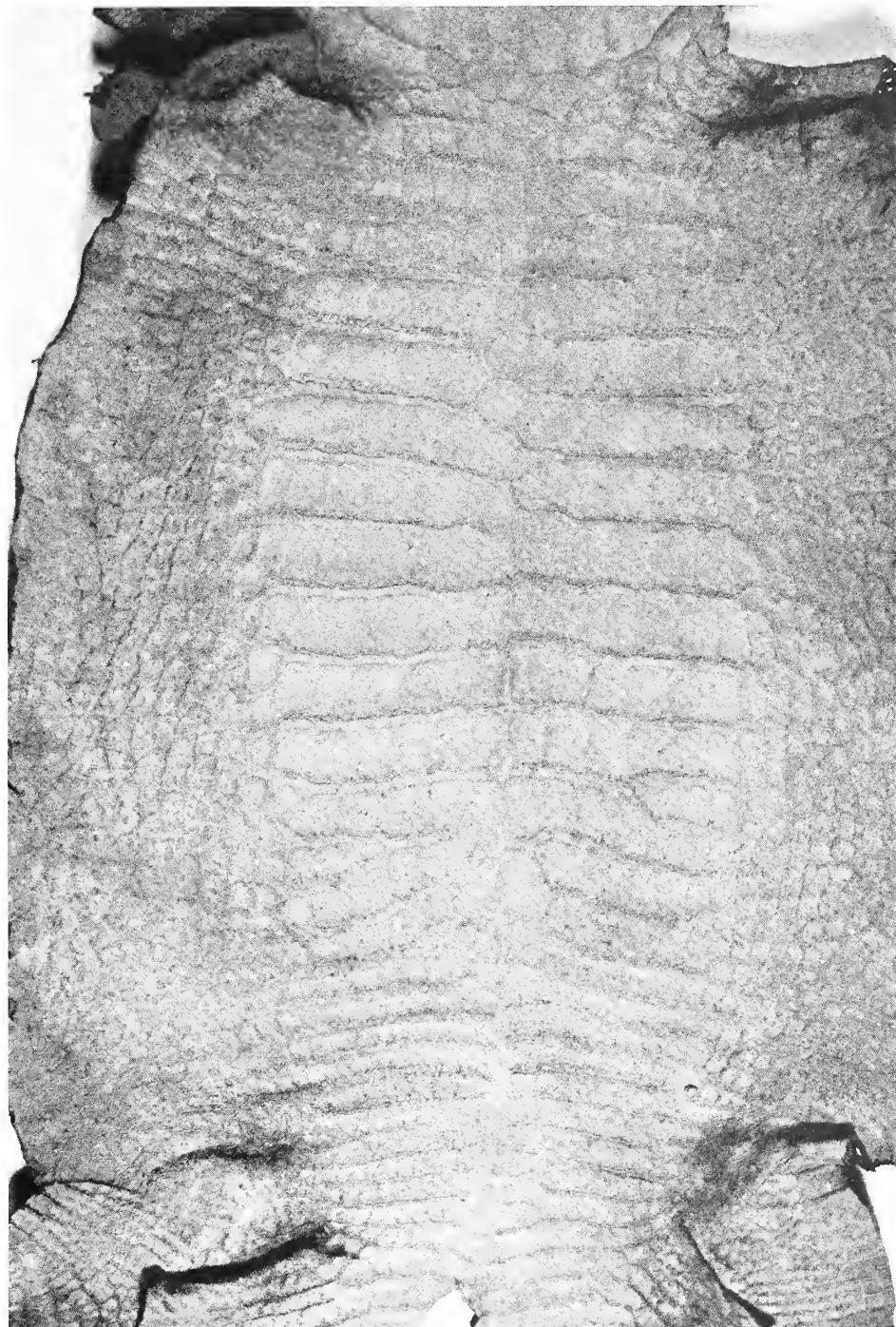
FIGURE 6. Inside surface of a finished adult American alligator (*Alligator mississippiensis*) belly hide. Note the total absence of osteoderm buttons, which indicates the specimen probably came from Louisiana. Compare the inside of the ventral collar, just visible at the top of the photograph, with figure 8.
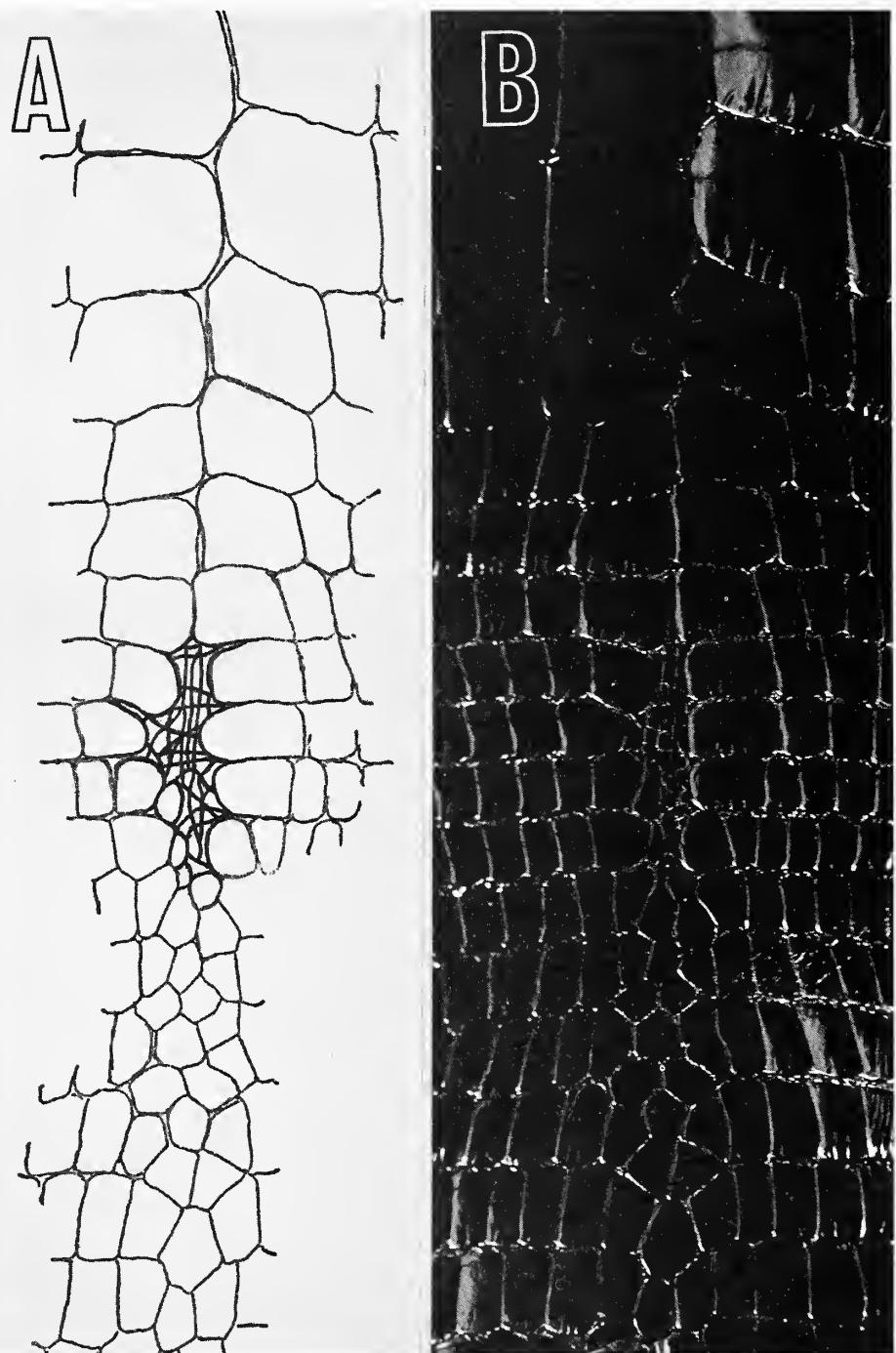
FIGURE 7. The spider-web umbilicus typical of American alligator (*Alligator mississippiensis*) belly hides — A is a diagrammatic illustration of the photograph B. Also note the absence of both surface pitting and follicle glands on the ventral scales.
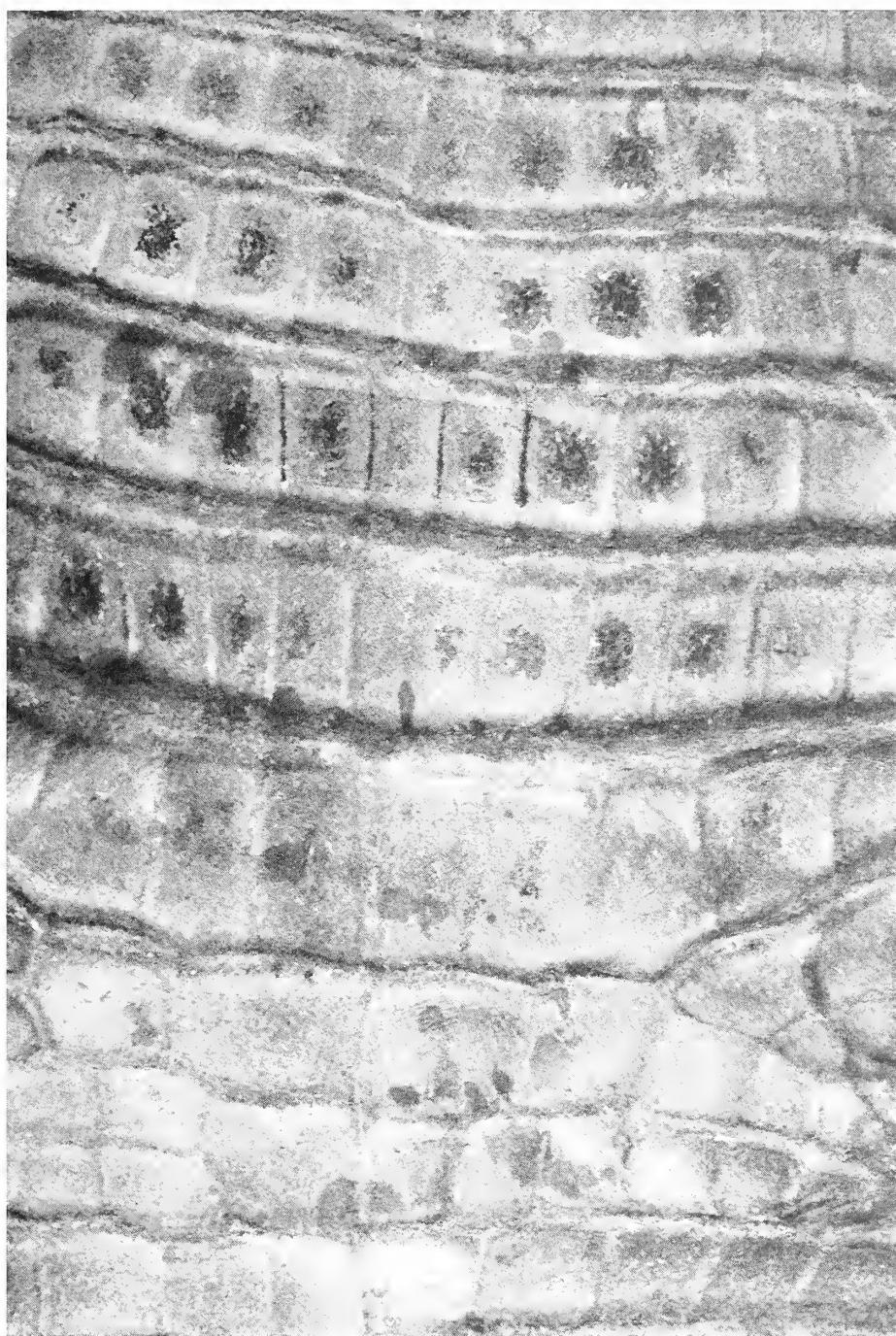
FIGURE 8. Inside surface of a finished American alligator (*Alligator mississippiensis*) hide from Florida. The portion shown is from the throat area as evidenced by the ventral collar. The dark round blotches in the center of the scales are single osteoderm buttons.
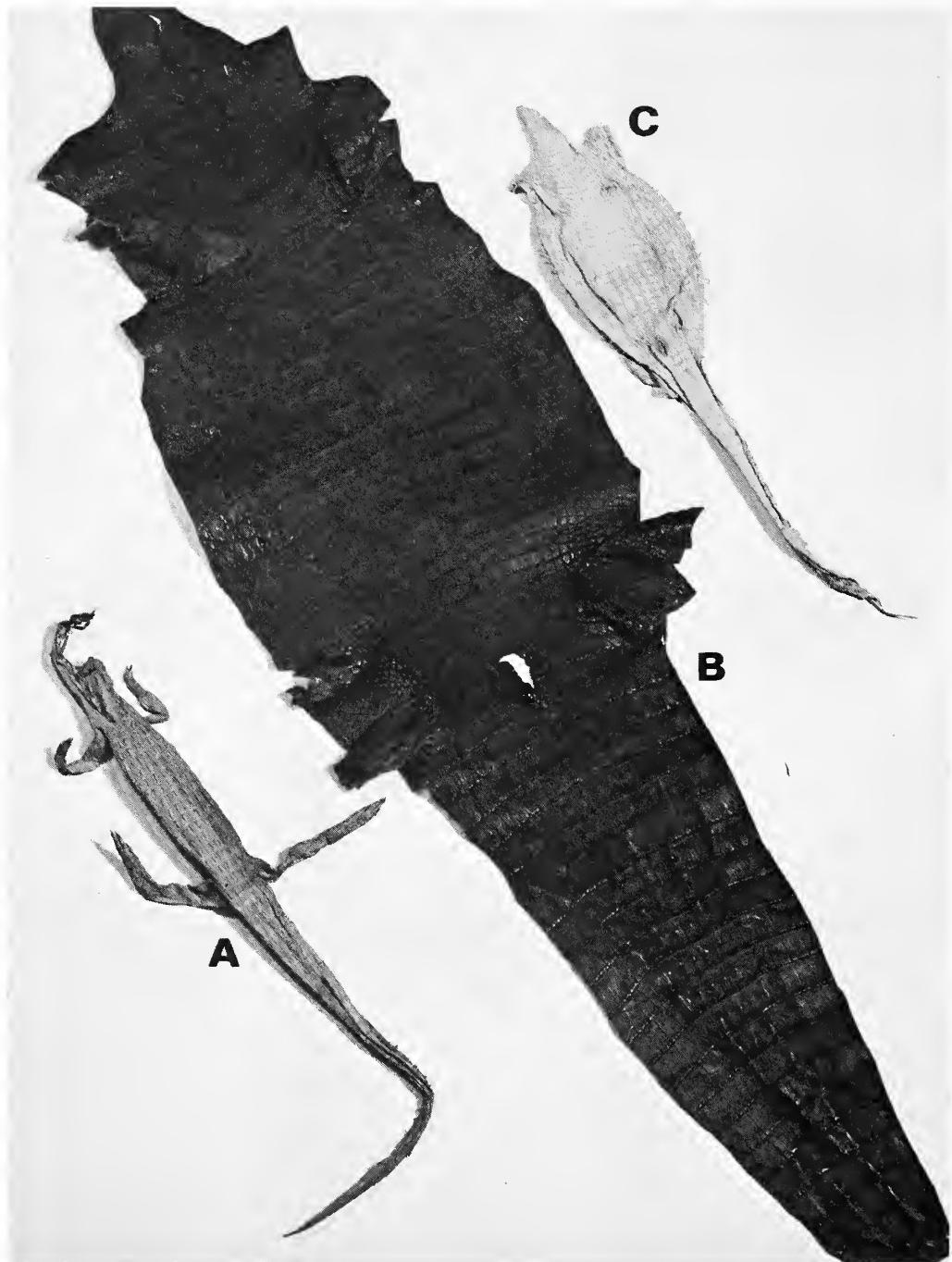
FIGURE 9. Hornback (A) and belly hides (B and C) of a South American caiman (*Caiman crocodilus*). Note the presence of the vent in both belly hides. A and C are crusts. B is a hide with *sauvage* finish.
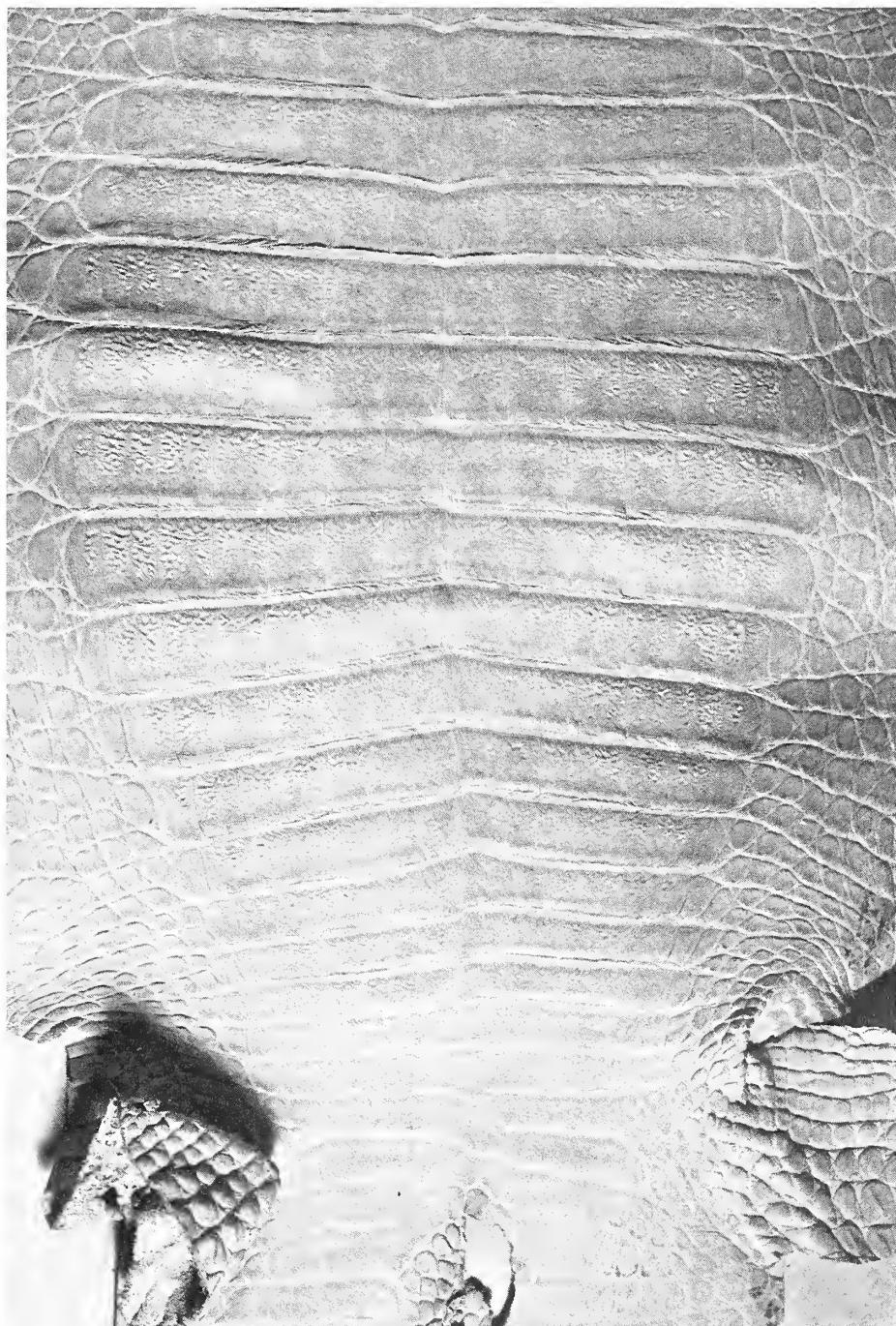
FIGURE 10. Outside surface of a crust belly hide of a South American caiman (*Caiman crocodilus*). Note the surface pitting which is indicative of underlying osteoderm buttons.
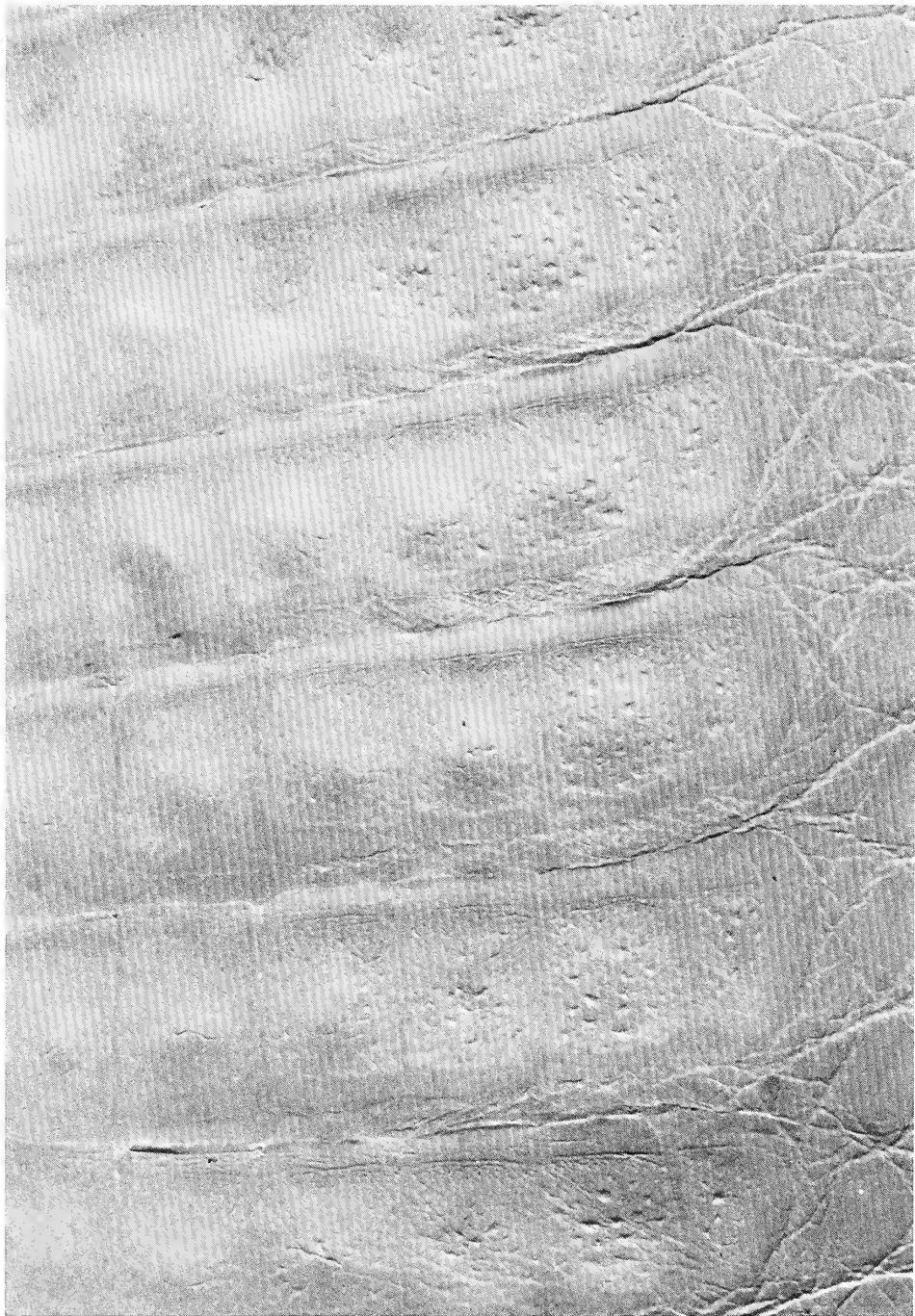
FIGURE 11. Ventral scales of a South American caiman (*Caiman crocodilus*) crust. Note the surface pitting. Lateral scales are just visible on the right side of the photograph.
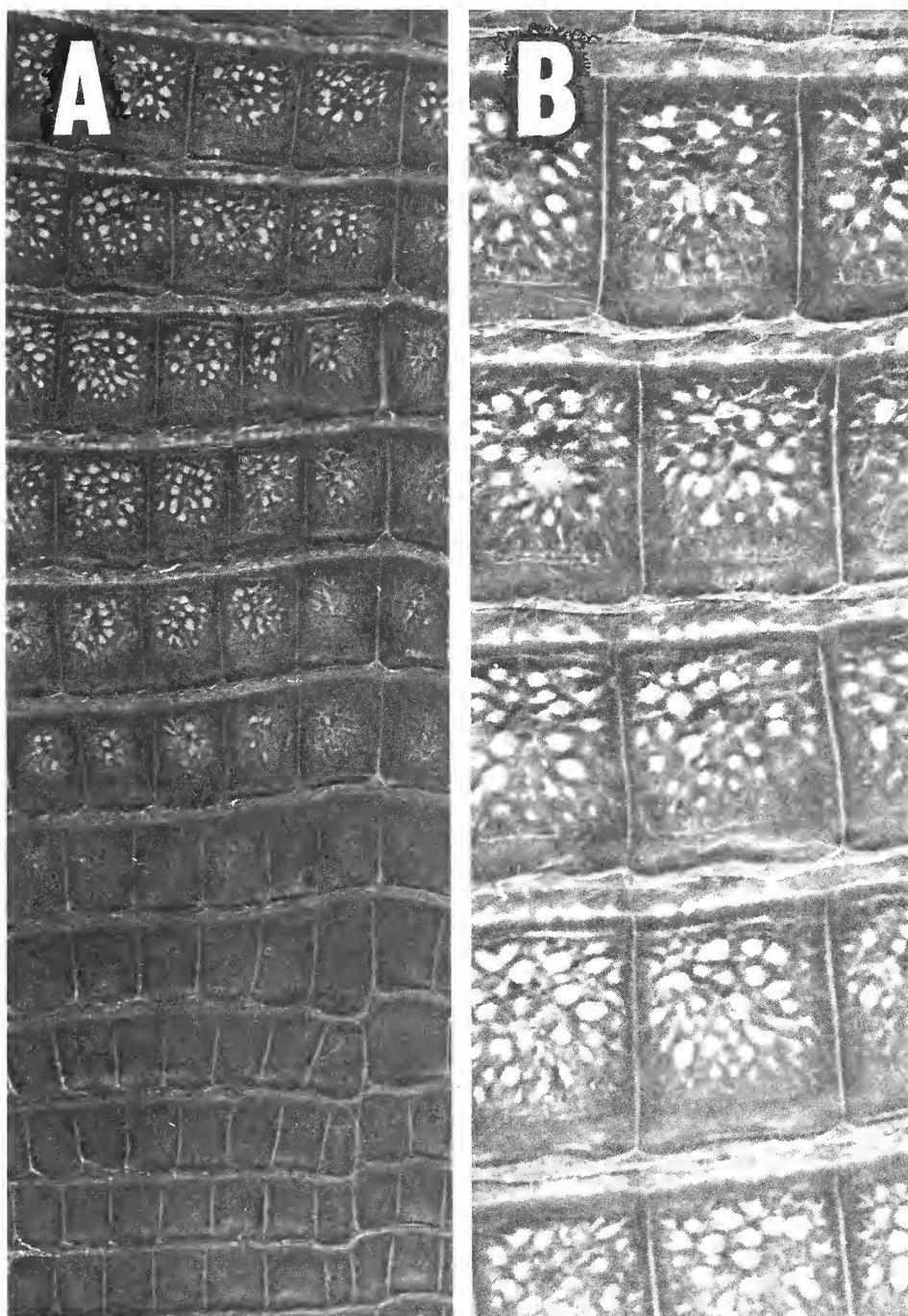
FIGURE 12. Ventral scales of a finished South American caiman (*Caiman crocodilus*) belly hide. Photograph B is a close view of the scales seen in A. Because of the technique used to dye this hide, the surface pits are white against a dark background. Note that the pitting is not as pronounced near the vent (lower half of A) as near midbelly (upper half of A).
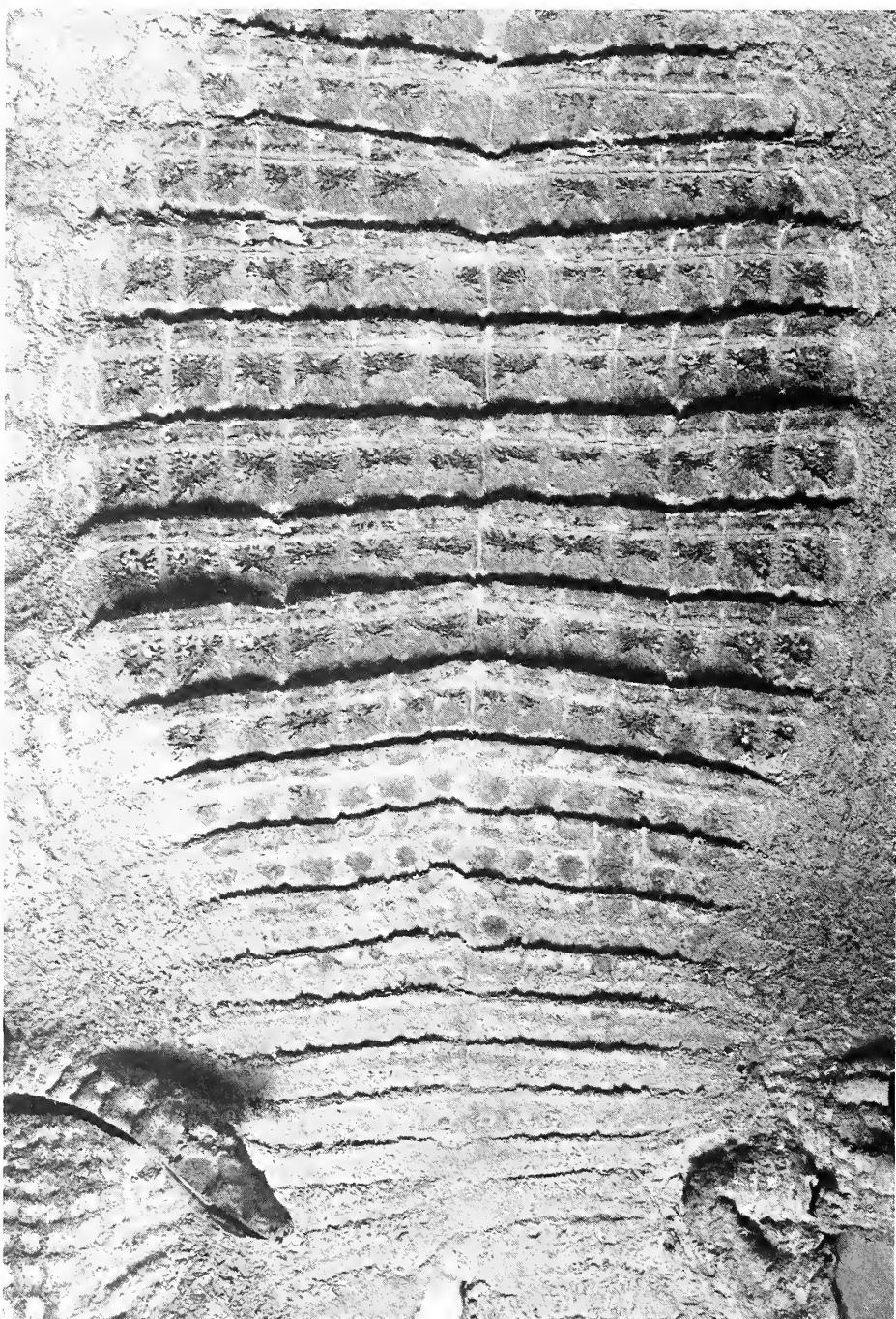
FIGURE 13. Inside surface of a South American caiman (*Caiman crocodilus*) crust. Note the presence of double osteoderm buttons in the ventral scales. Closer views are provided in figure 14.
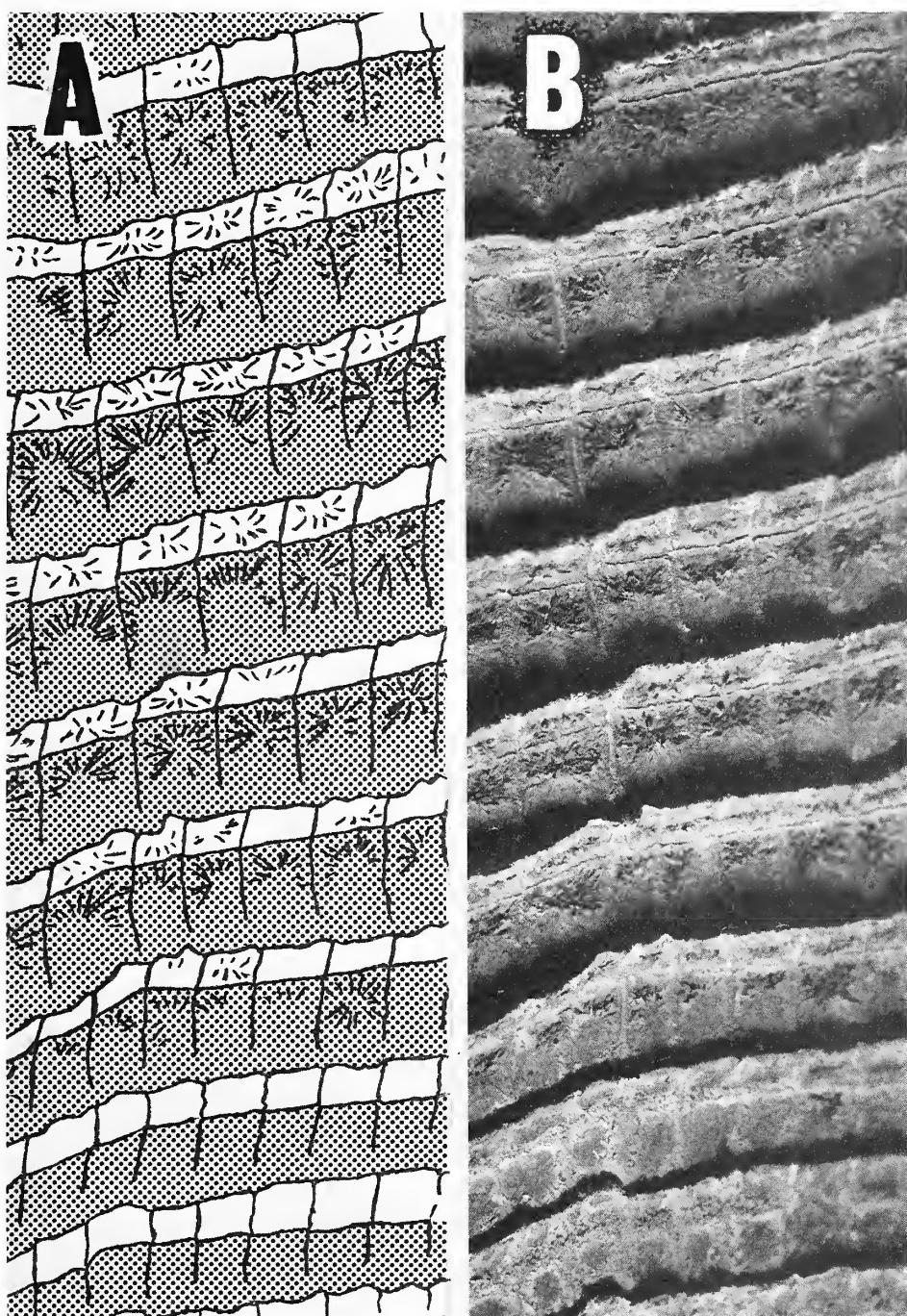
FIGURE 14. Double osteoderm buttons on the inside surface of a South American caiman (*Caiman crocodilus*) belly crust. A is a diagrammatic illustration of the photograph B. Each ventral scale contains two osteoderms, double buttons. The larger posterior button is shaded in A, while the smaller inward-curving anterior button is unshaded. Most of the anterior button is removed when the hide is shaved. Compare this figure with the shaved hide in figure 19.
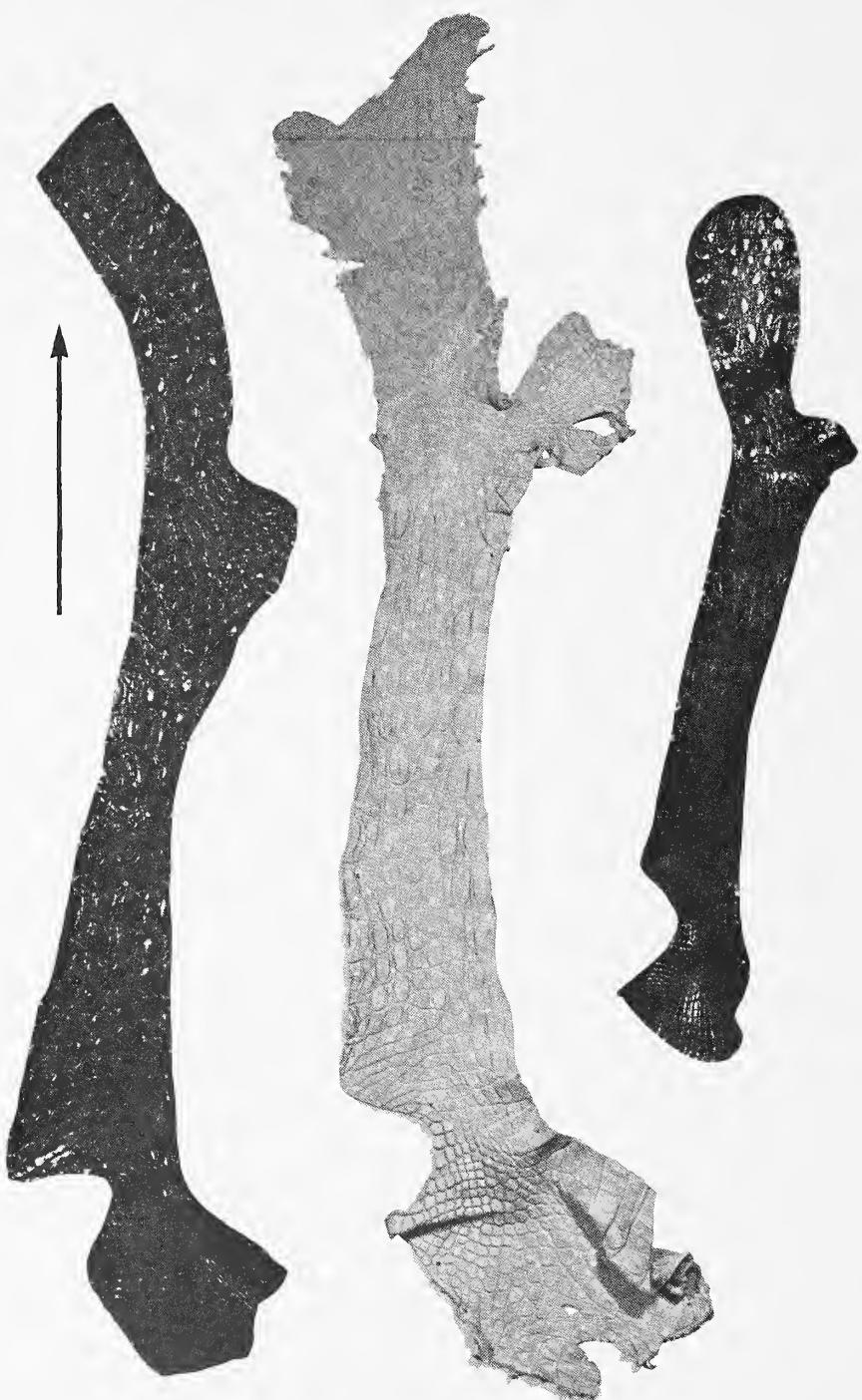
FIGURE 15. Sides of South American caiman (*Caiman crocodilus*). The center hide is a crust. The other two are finished hides. The arrow indicates the anterior (cephalic) end of the hide.
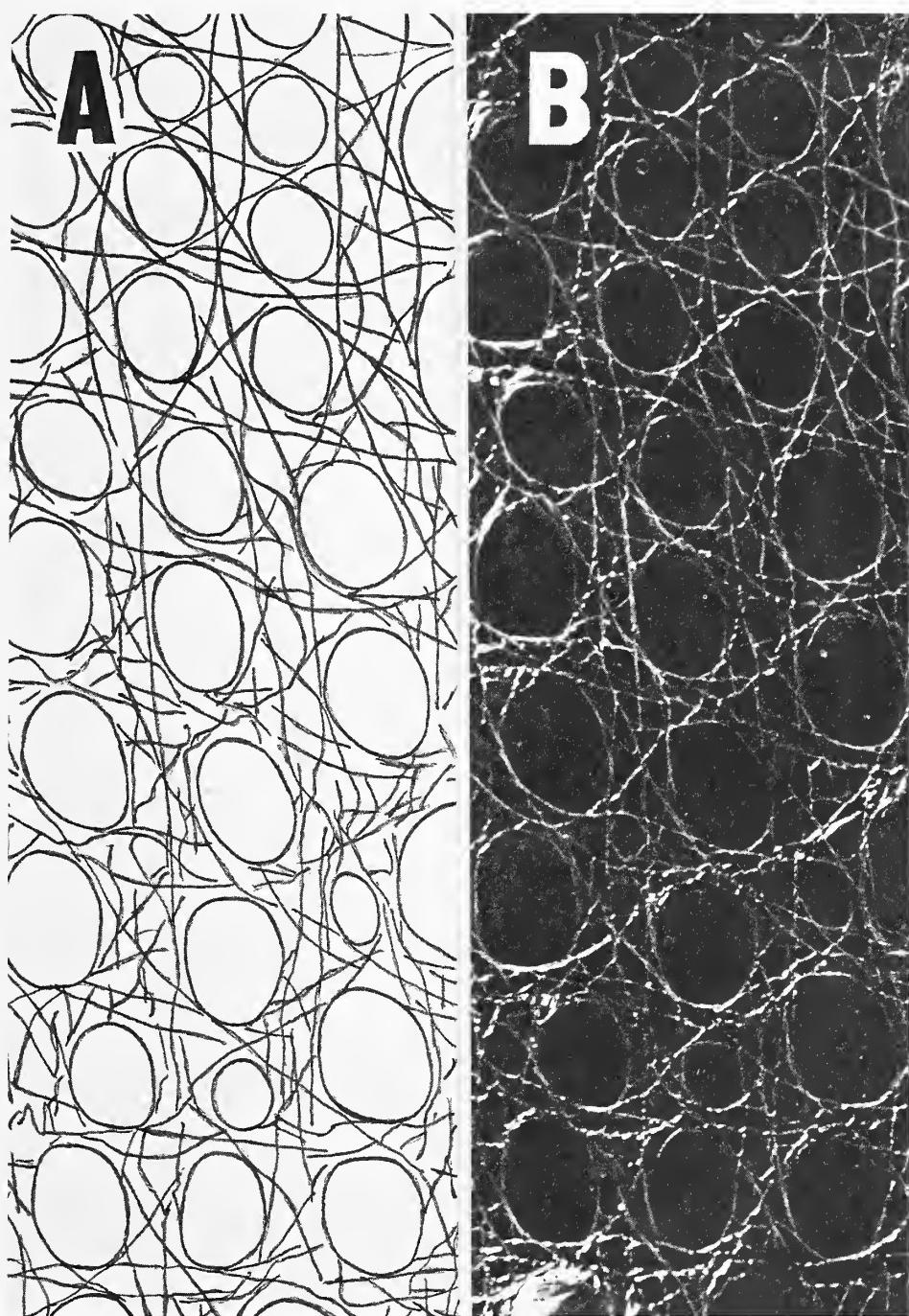
FIGURE 16. Scales of finished South American caiman (*Caiman crocodilus*) sides. A is a diagrammatic illustration of photograph B. Note that the rows of large oval scales alternate with strips of soft skin with a network of creases. Compare this with figure 21.
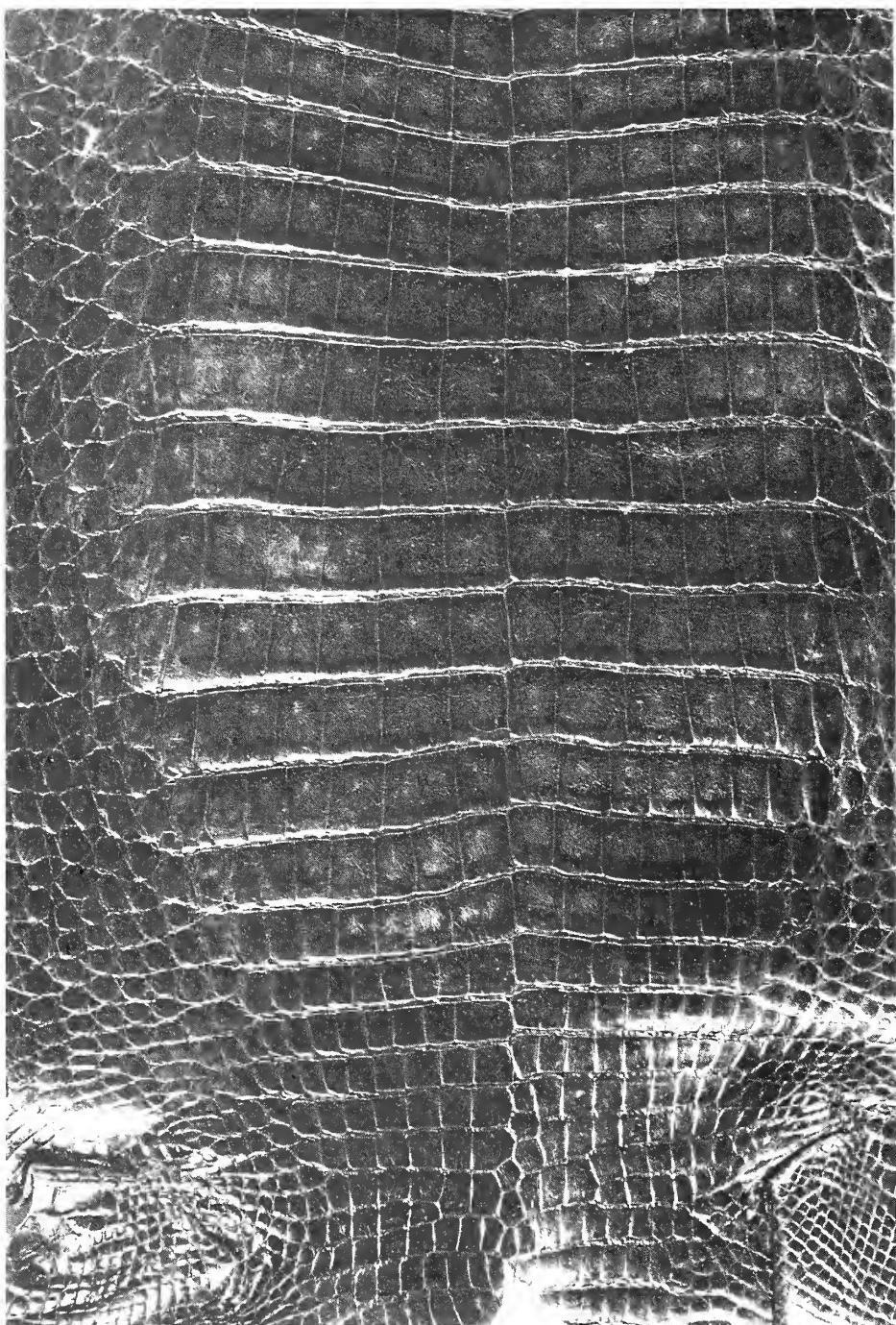
FIGURE 17. Outside surface of a finished black caiman (*Melanosuchus niger*) belly hide. A closer view of the ventral scales is provided in figure 18.
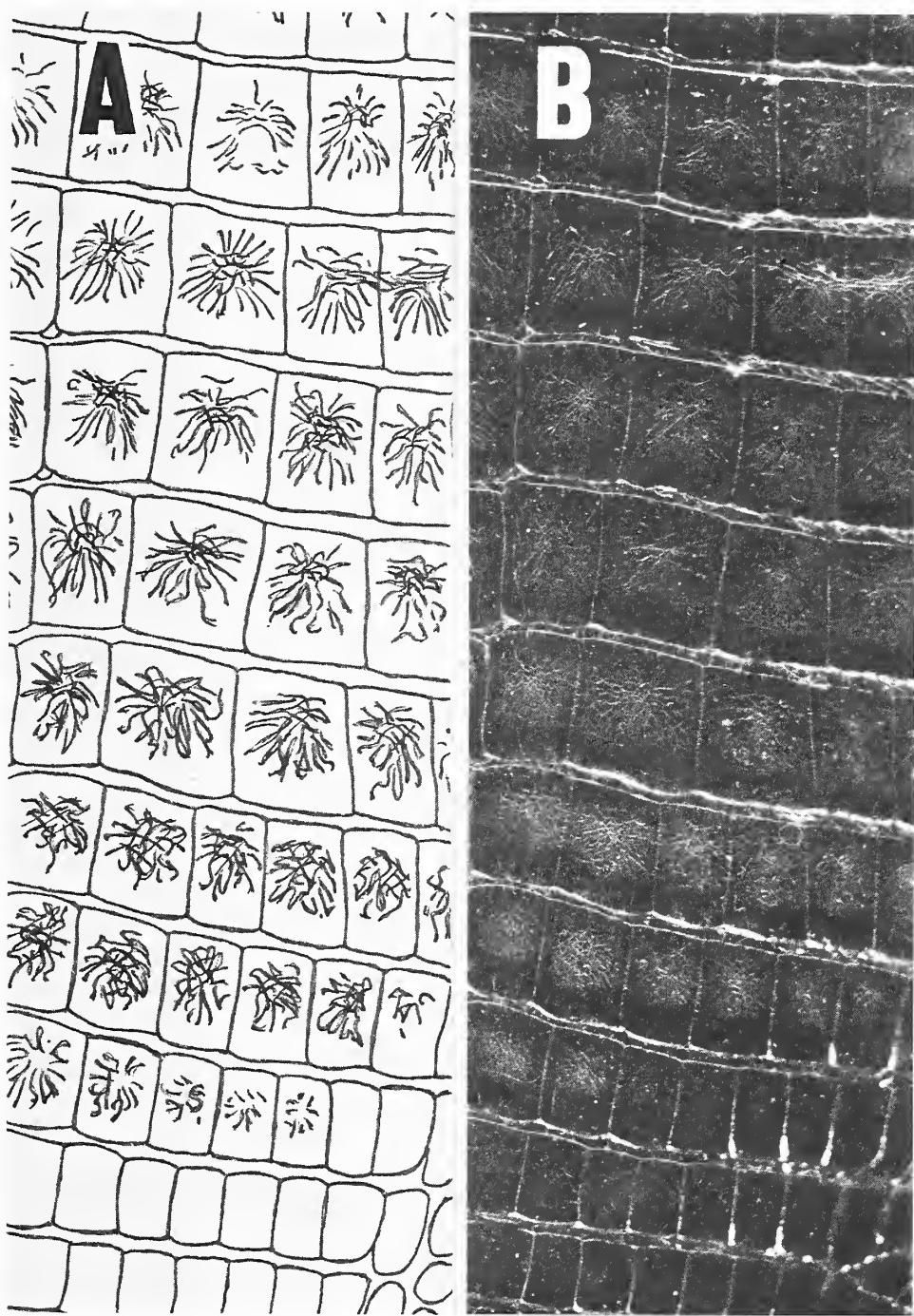
FIGURE 18. Ventral scales of a finished black caiman (*Melanosuchus niger*) belly hide. A is a diagrammatic illustration of the photograph B. Note the wrinkles and fine surface pitting, as well as the lighter color in the centers of the scales. Both conditions are indicative of underlying osteoderm buttons.
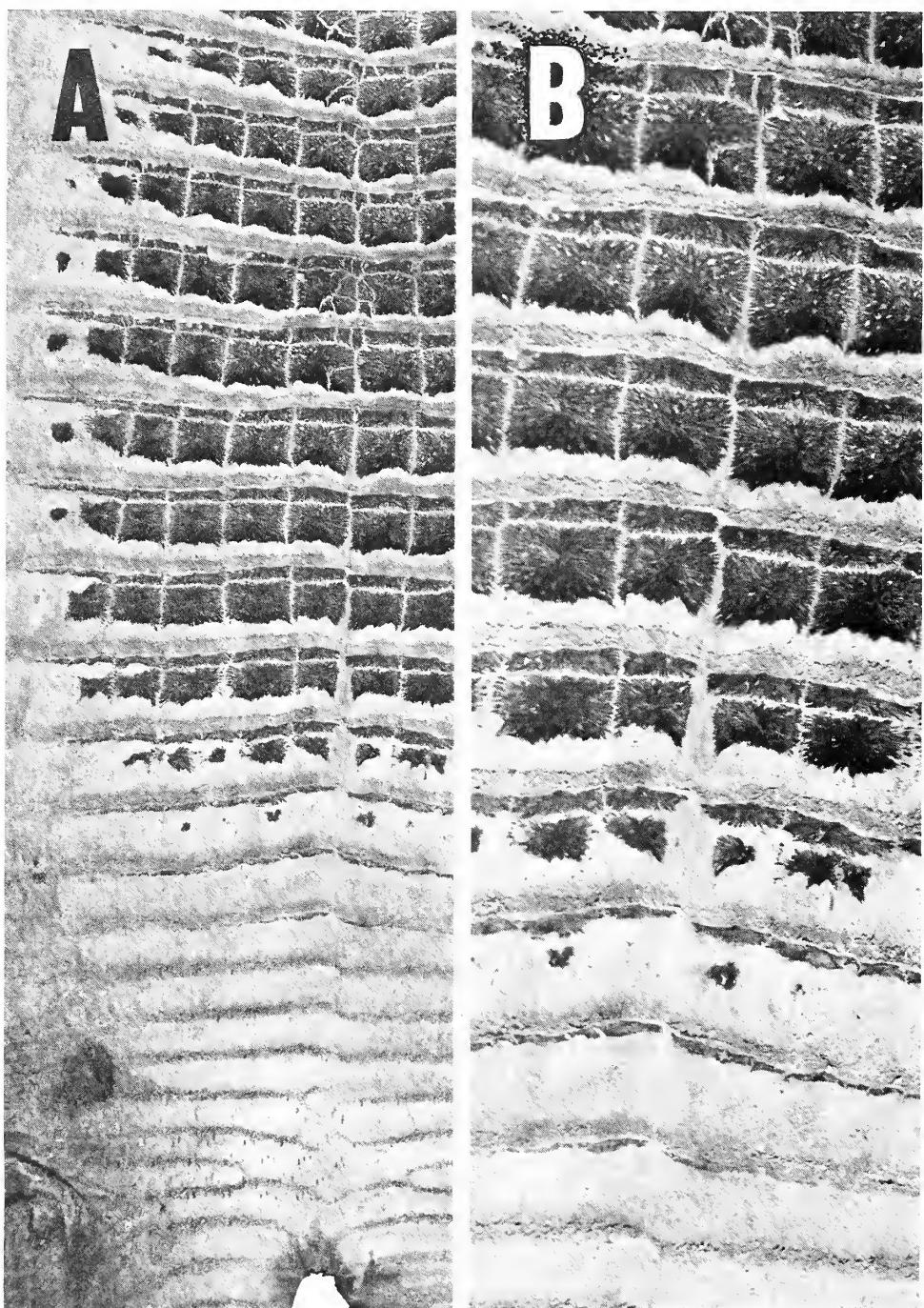
FIGURE 19. Inside surface of a finished black caiman (*Melanosuchus niger*) belly hide. Note the dark double osteoderm buttons in each scale. Photograph B is a close view of the buttons seen in A. This hide has been shaved so most of the anterior button has been removed. Compare it with figures 13 and 14.
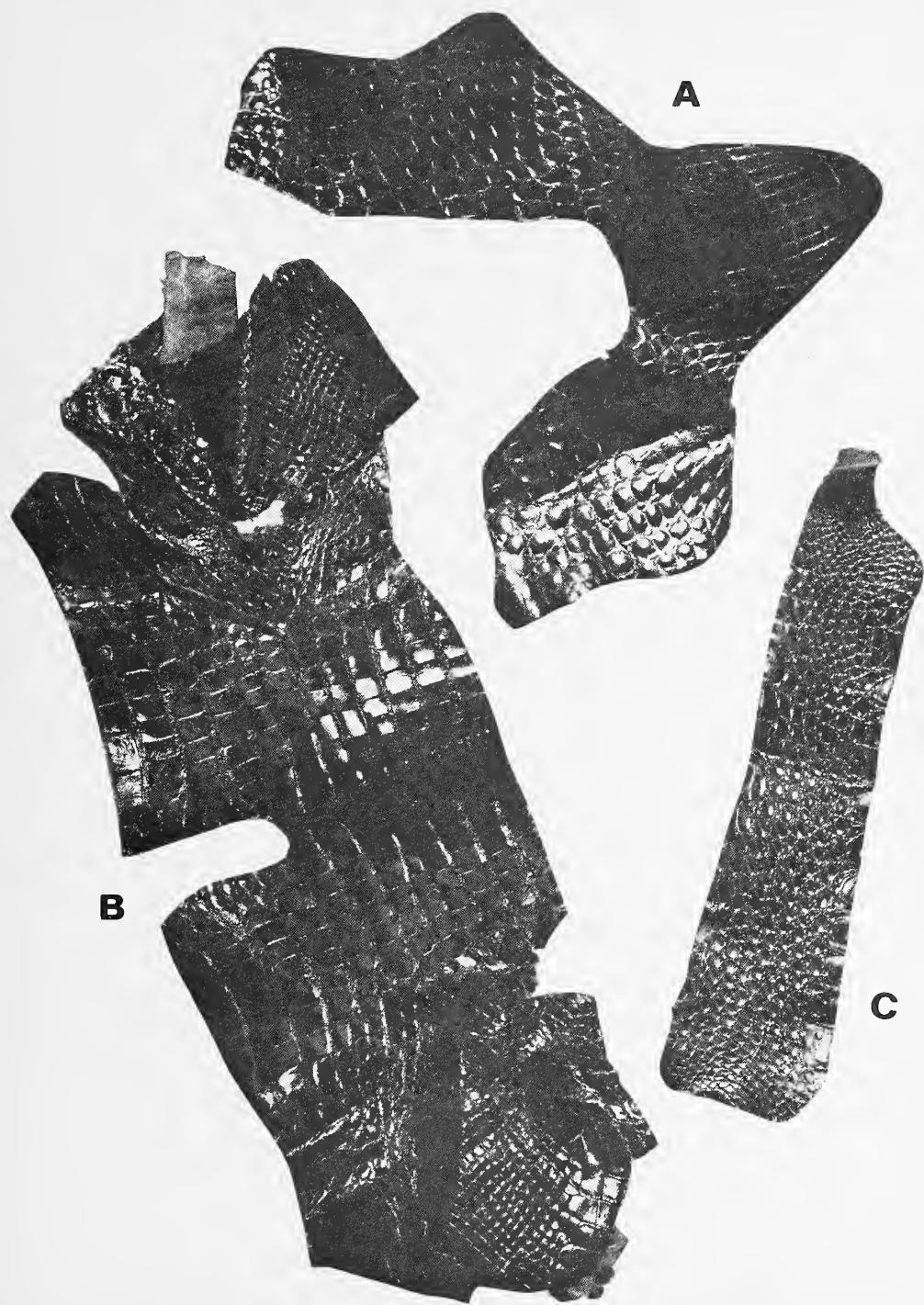
FIGURE 20. Outside surface of finished black caiman (*Melanosuchus niger*) throat (A), girdle (B), and side (C). The scales of the side are shown in figure 21.
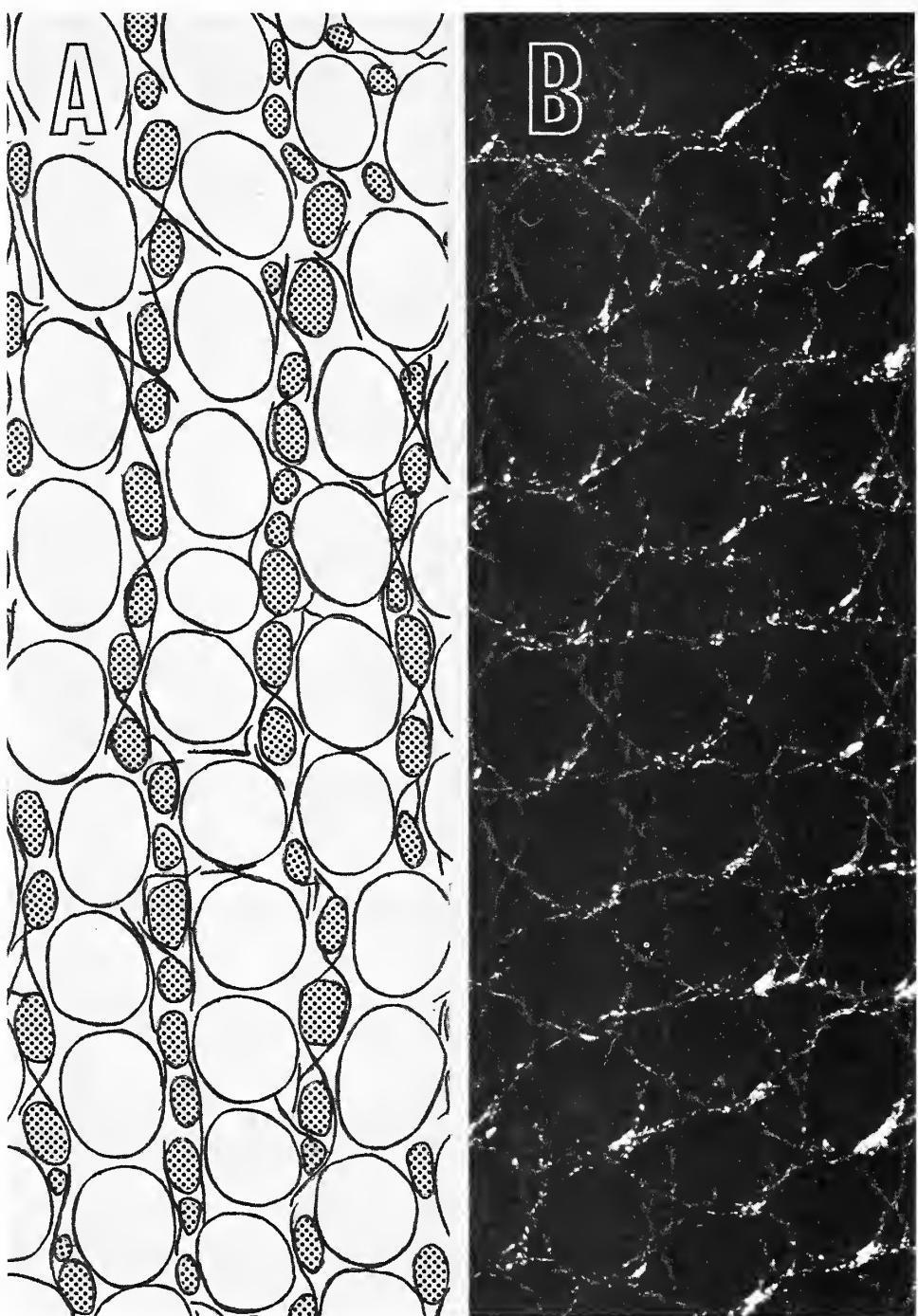
FIGURE 21. Scales of finished black caiman (*Melanosuchus niger*) side. A is a diagrammatic illustration of photograph B. Note that the large oval scales alternate with rows of small scales. Compare this with figure 16.
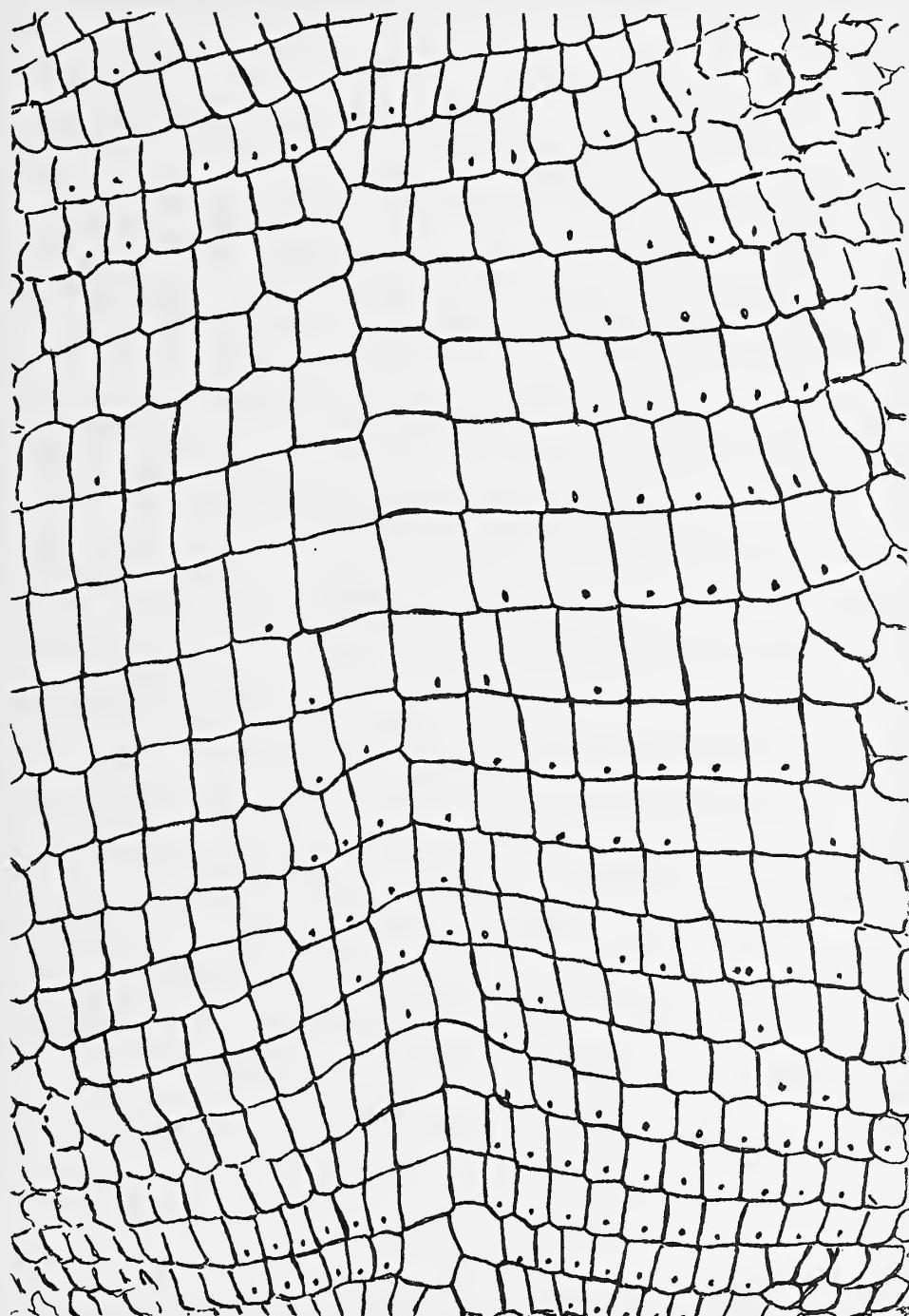
FIGURE 22. Diagrammatic illustration of the Nile crocodile belly hide shown in figure 23. Note the presence of follicle glands (only those visible in figure 23 are illustrated).
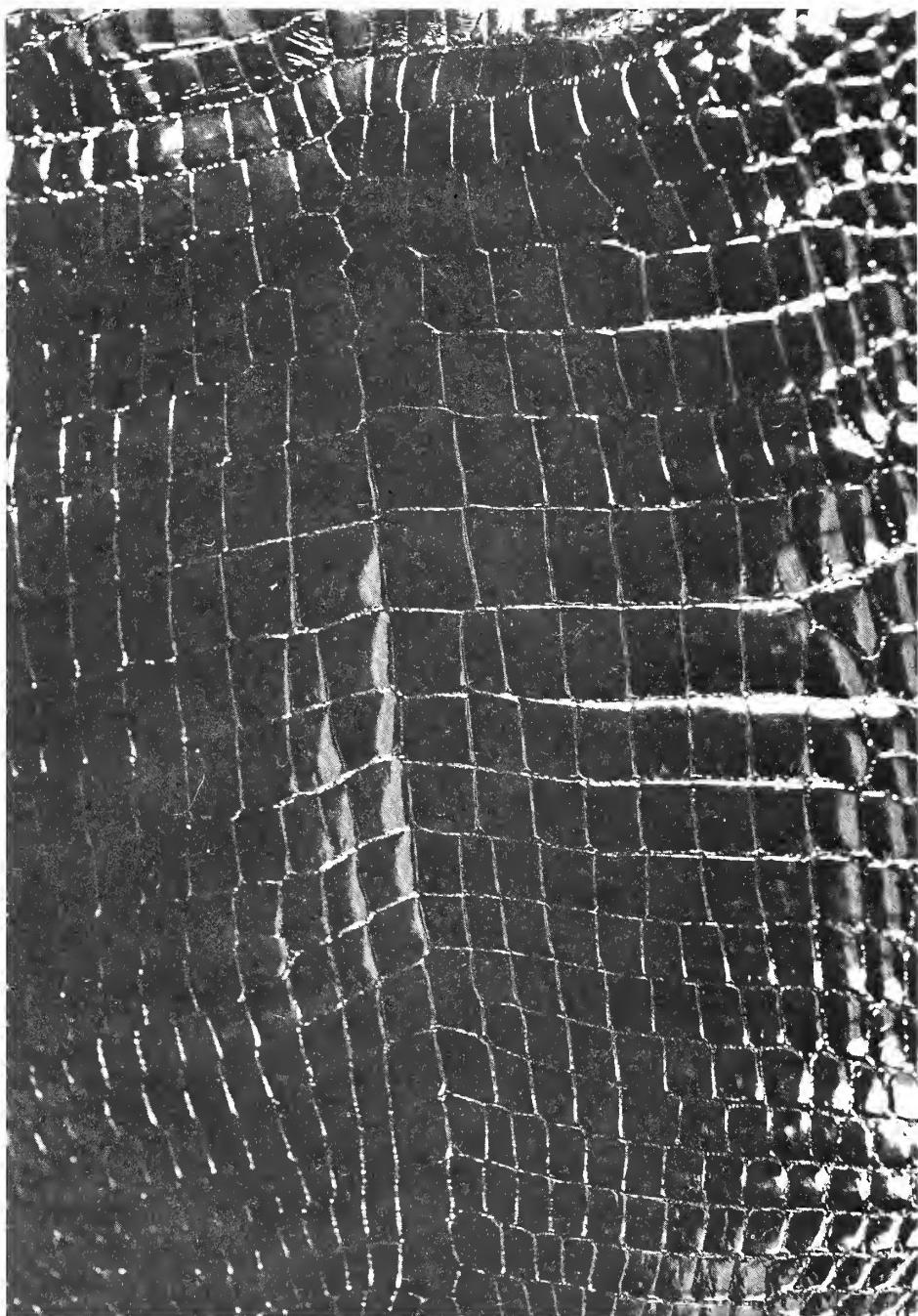
FIGURE 23. Outside surface of a finished Nile crocodile (*Crocodylus niloticus*) belly hide. Compare it with figure 22.
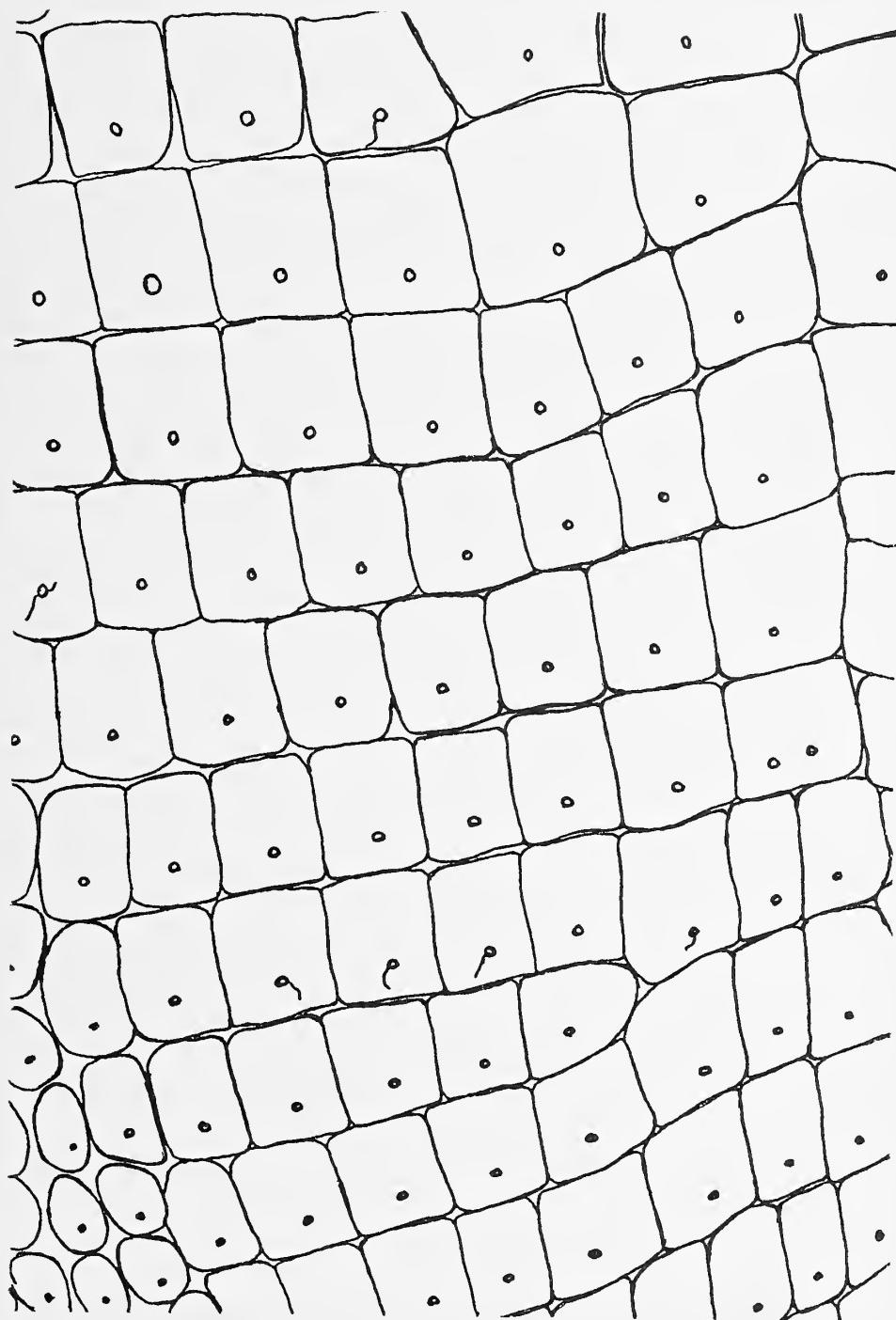
FIGURE 24. Diagrammatic illustration of the ventral scales of the Morelet's crocodile hide shown in figure 25. Note the prominent follicle glands.
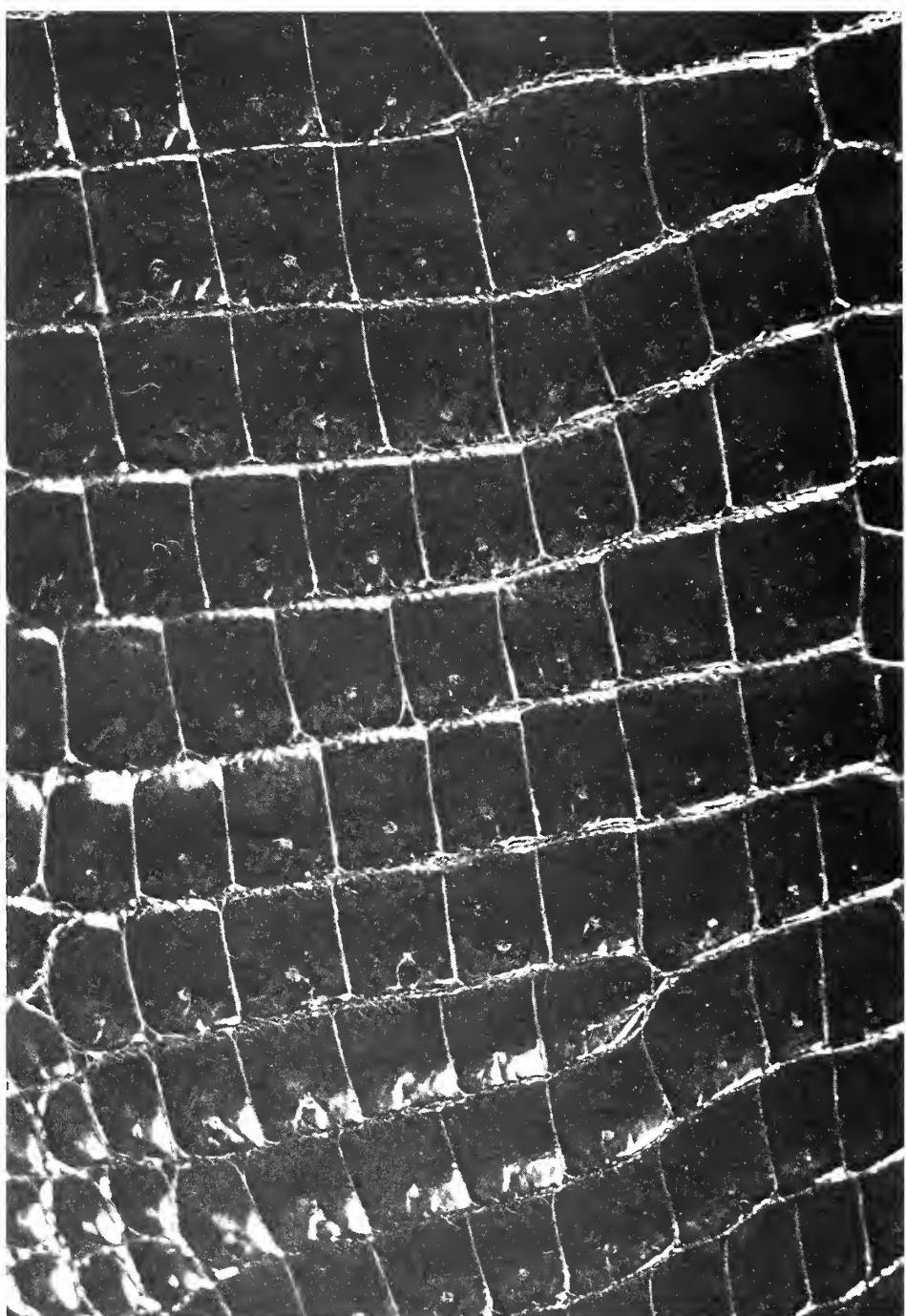
FIGURE 25. Ventral scales of a finished Morelet's crocodile (*Crocodylus moreletii*) belly hide. Compare it with figure 24.
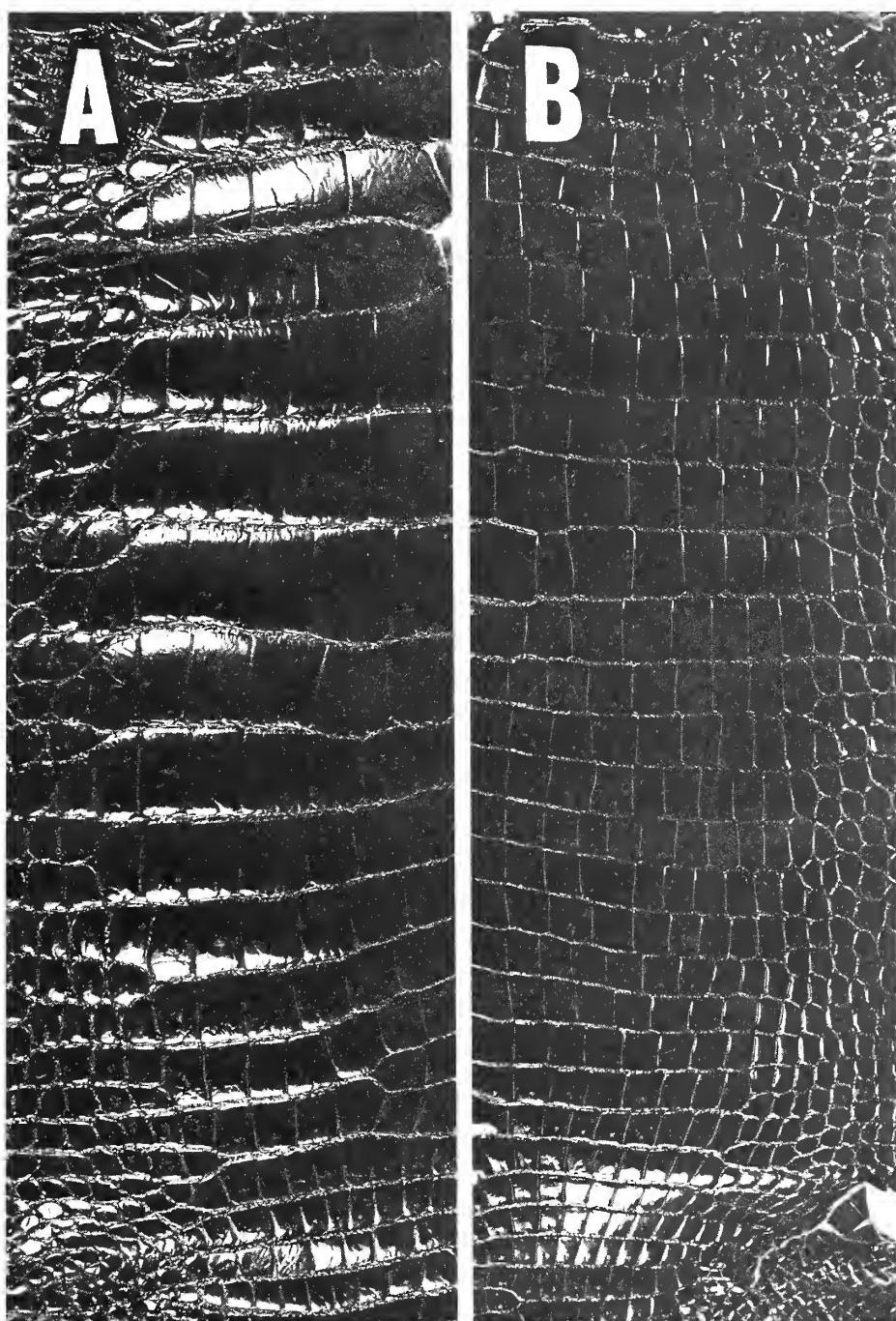
FIGURE 26. Comparison of scale size on (A) large scale false gavial (*Tomistoma schlegelii*) and (B) small scale saltwater crocodile (*Crocodylus porosus*) belly hides.
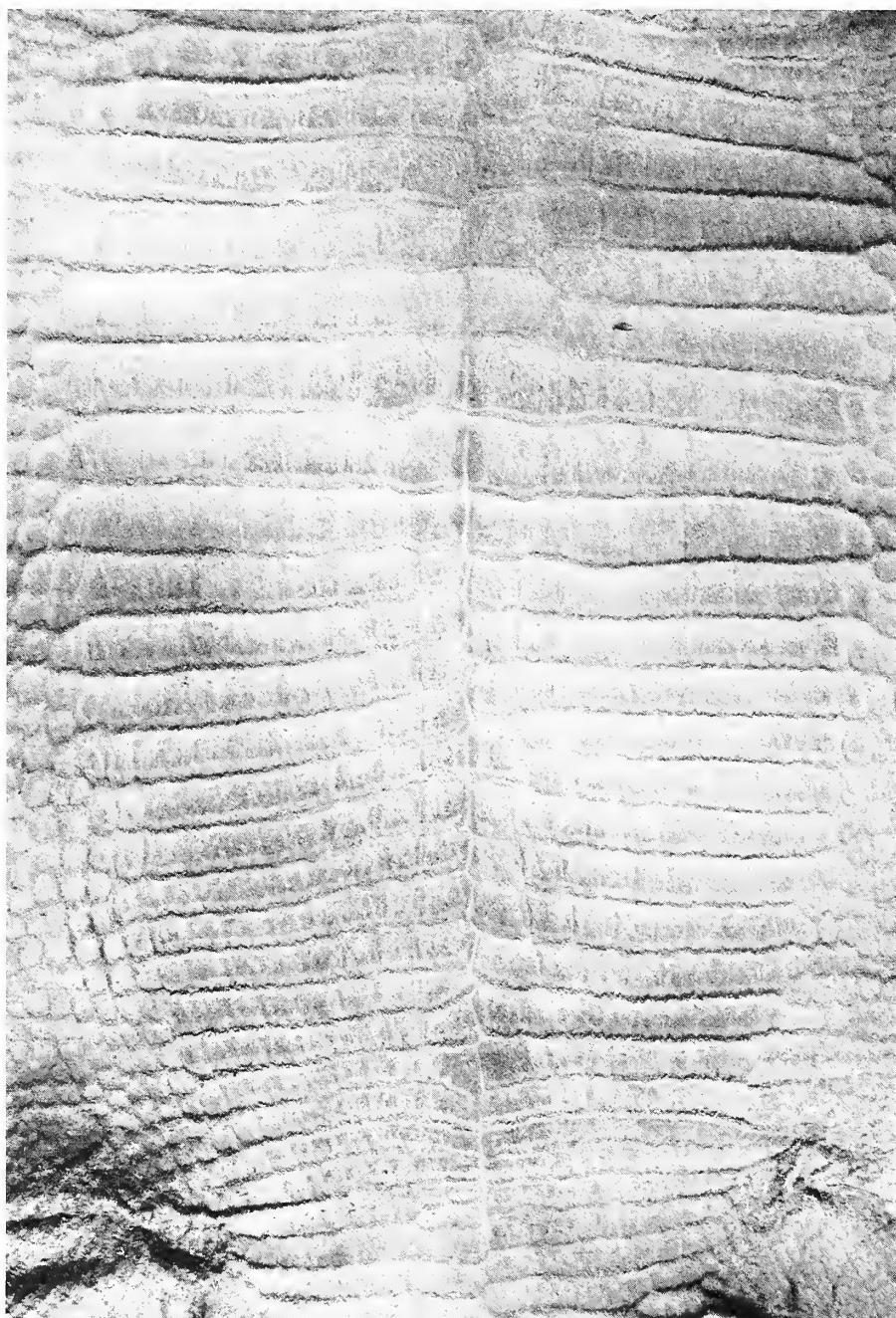
FIGURE 27. Inside surface of a finished saltwater crocodile (*Crocodylus porosus*) belly hide. Note the total absence of osteoderm buttons.
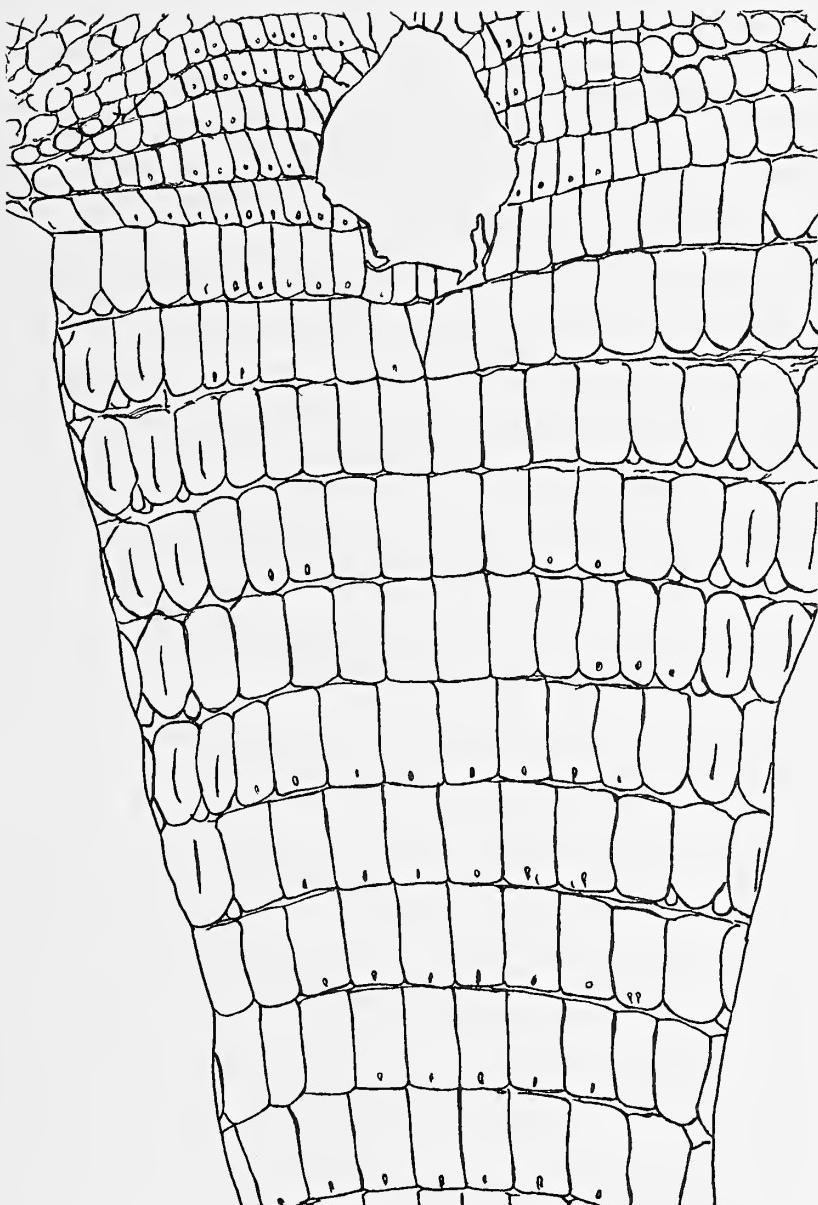
FIGURE 28. Diagrammatic illustration of the Nile crocodile tail whorls shown in figure 29. Note both the presence of follicle glands (only the ones visible in figure 29 are illustrated) and the regular arrangement of the whorls. Compare it with figure 30.

FIGURE 29. Tail whorls of a finished Nile crocodile (*Crocodylus niloticus*) belly hide. Compare it with figure 28.
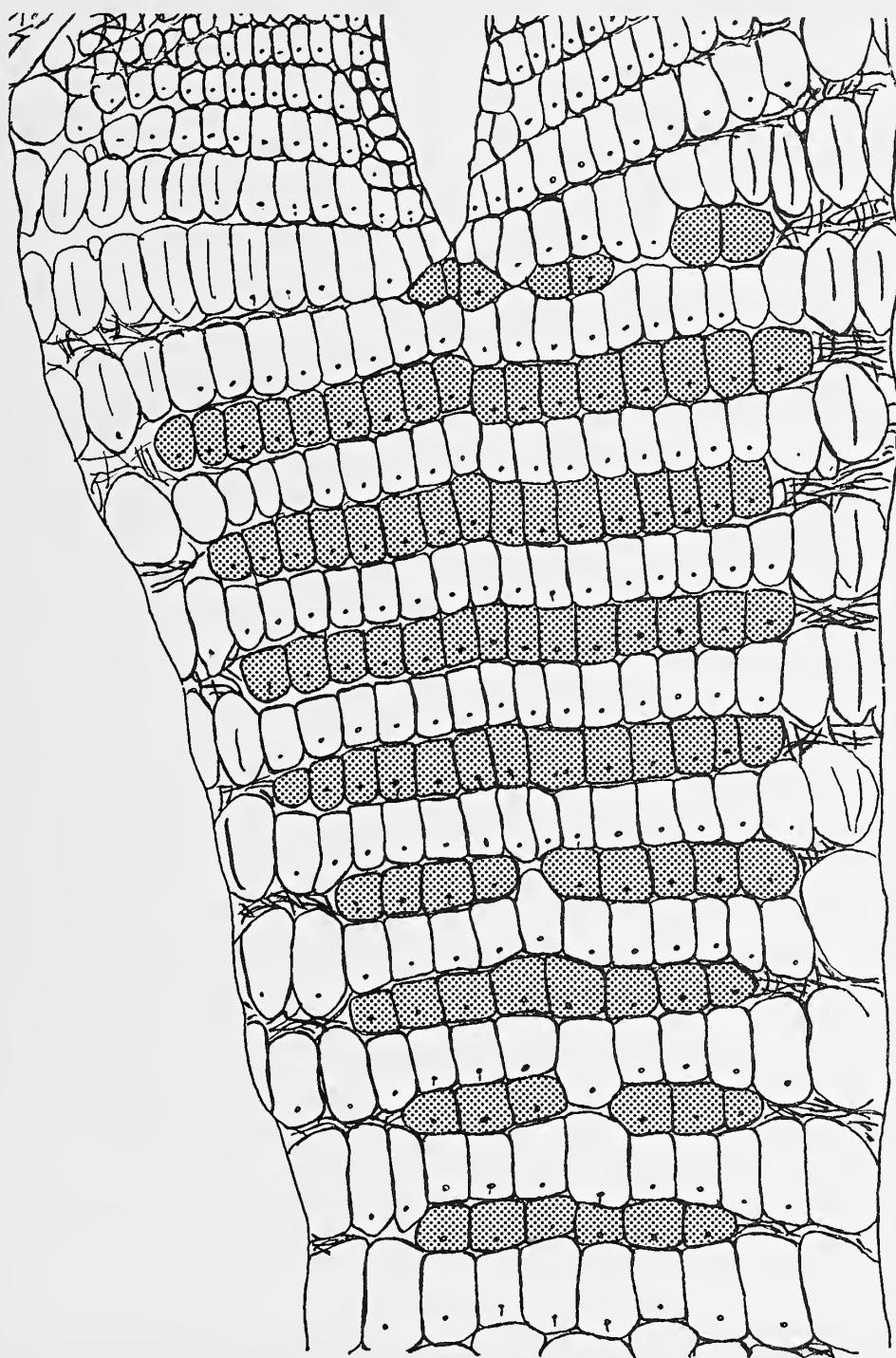
FIGURE 30. Diagrammatic illustration of the Morelet's crocodile tail whorls shown in figure 31. Note the presence of both follicle glands and irregular and incomplete (shaded) whorls. Compare it with figure 28.
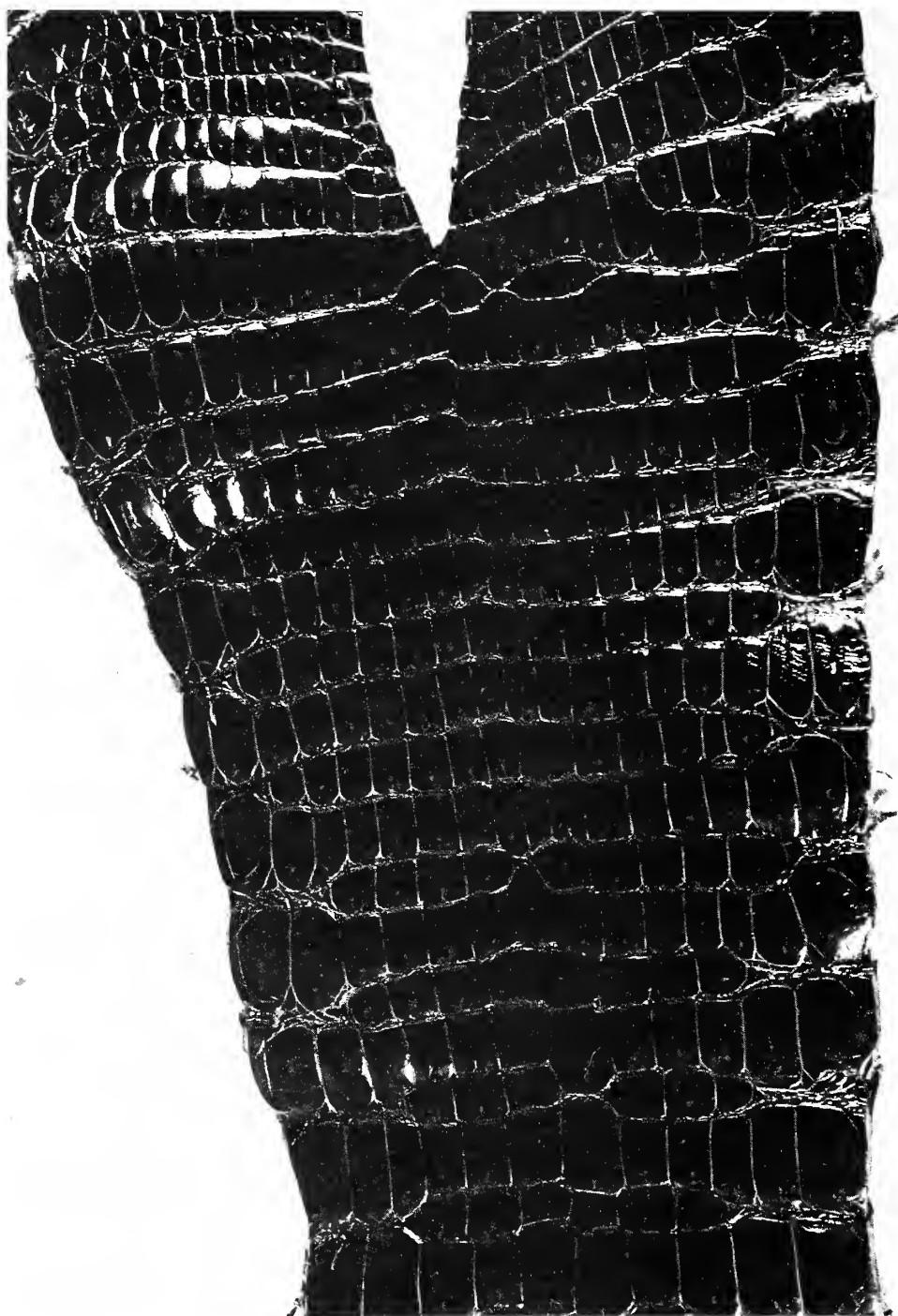
FIGURE 31. Tail whorls of a finished Morelet's crocodile (*Crocodylus moreletii*) belly hide. Compare it with figure 30.
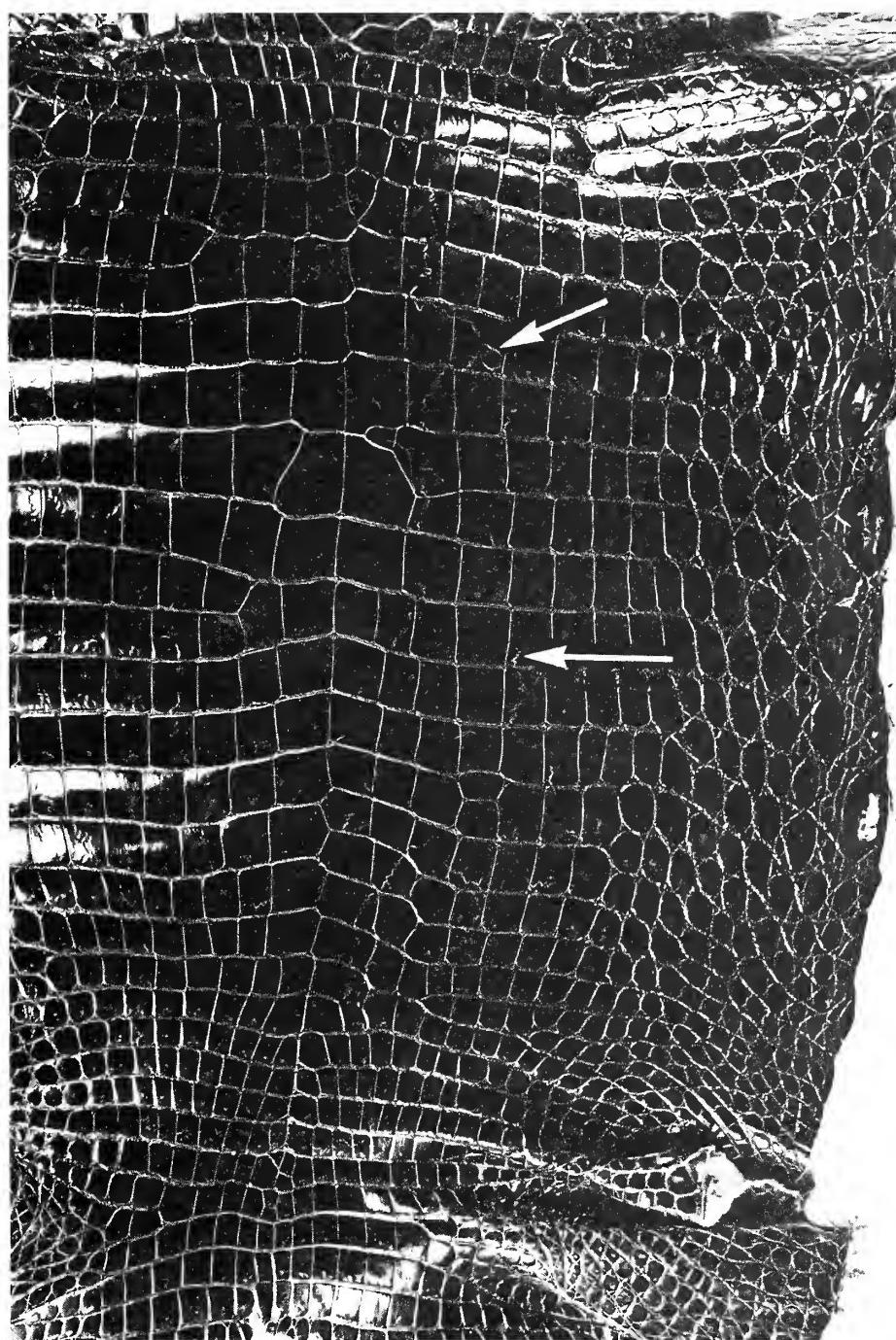
FIGURE 32. Outside surface of a finished Orinoco crocodile (*Crocodylus intermedius*) hide. The arrows indicate the location of parasitic "worm trails." Close views of these trails are shown in figure 33.

FIGURE 33. Undulating "worm trails" on the ventral scales of an Orinoco crocodile (*Crocodylus intermedius*) belly hide. Similar trails have been seen on Johnson's crocodiles (*C. johnsoni*), Morelet's crocodiles (*C. moreletii*), Nile crocodiles (*C. niloticus*), and saltwater crocodiles (*C. porosus*). They probably occur on other species as well.
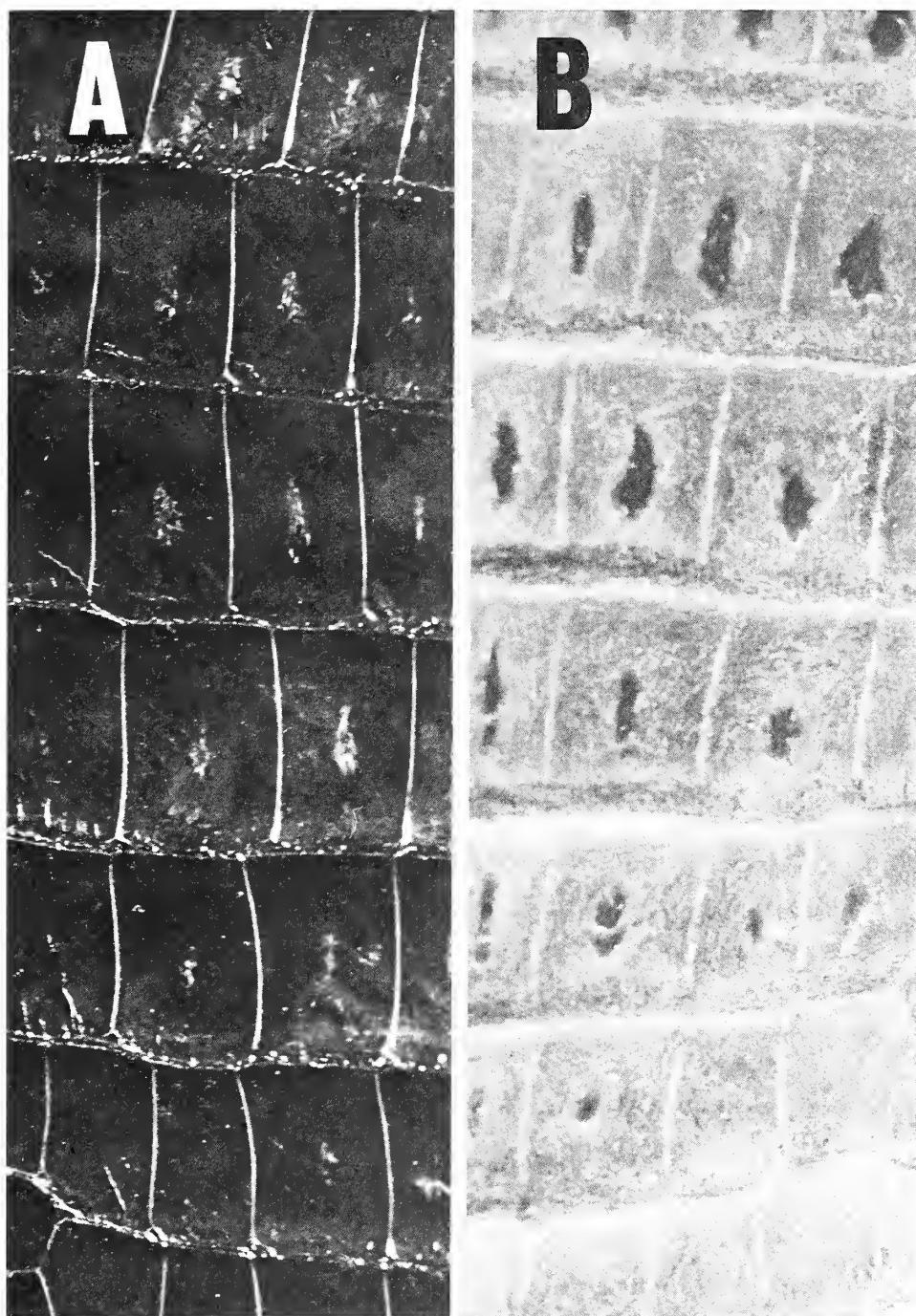
FIGURE 34. Outside (A) and inside (B) surfaces of finished ventral scales of either the African slender-snouted crocodile (*Crocodylus cataphractus*), Nile crocodile (*Crocodylus niloticus*), or dwarf crocodile (*Osteolaemus tetraspis*). Note the lighter color in the center of the scales (A), which are indicative of the underlying dark single osteoderm buttons (B).
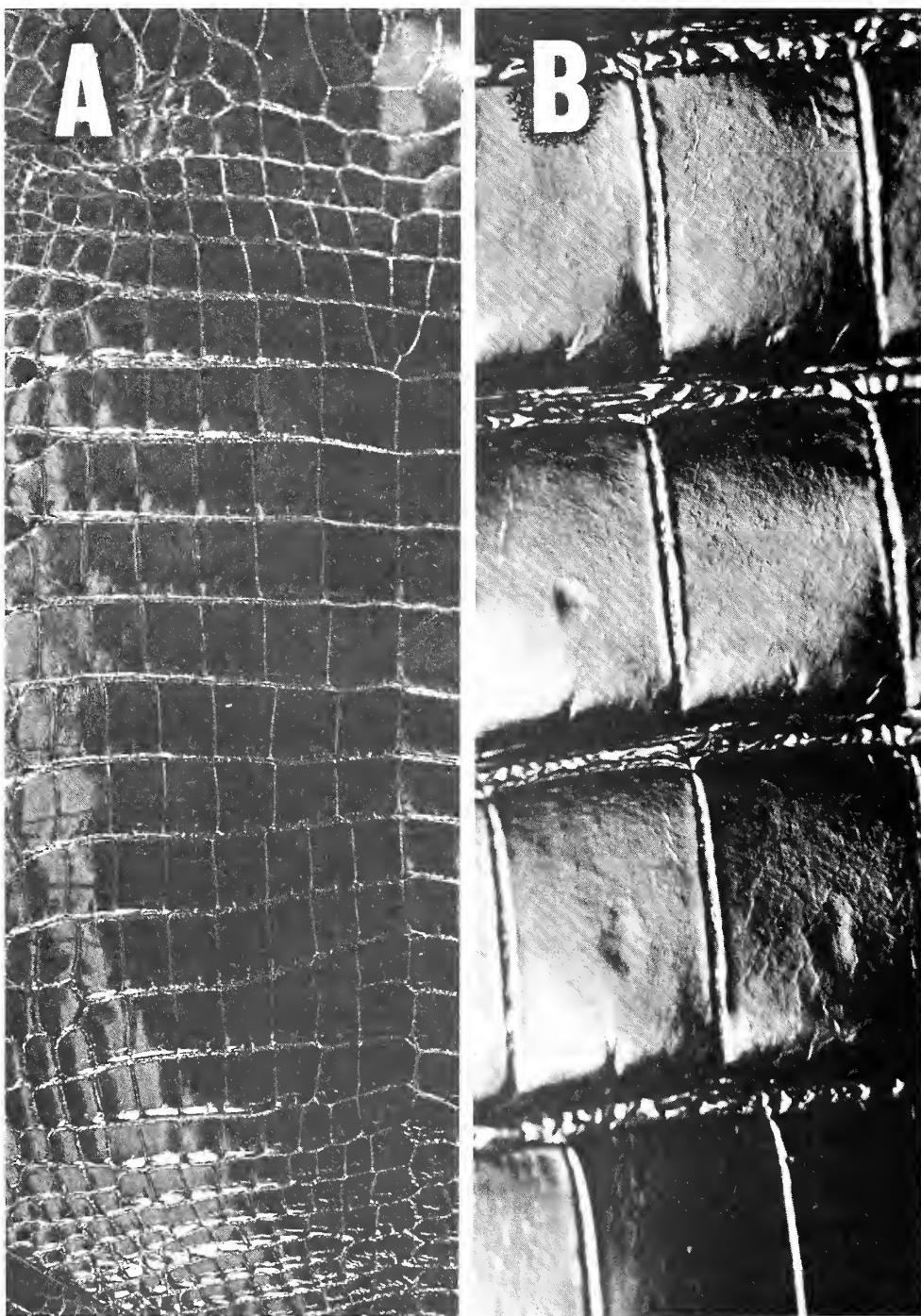
FIGURE 35. Outside surface of a finished belly skin (A) and ventral scales (B) of either an African slender-snouted crocodile *(Crocodylus cataphractus)*, Nile crocodile *(Crocodylus niloticus)* or dwarf crocodile *(Osteolaemus tetraspis)*. Note the shallow indentations, surface pits, indicative of underlying single buttons. Also note that follicle glands are reduced to deep wrinkles by polishing process.
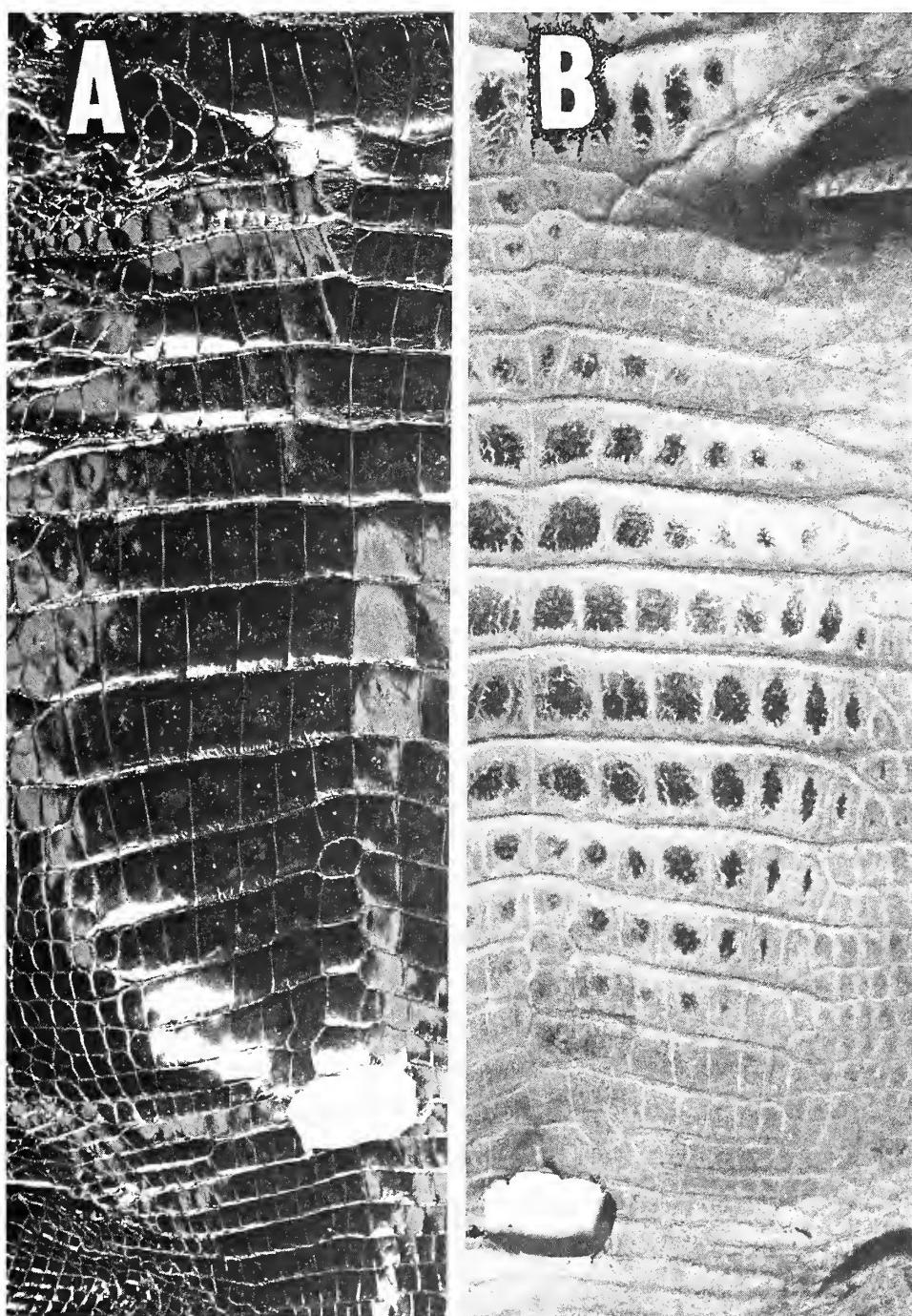
FIGURE 36.   Outside (A) and inside (B) surfaces of finished African slender-snouted crocodile *(Crocodylus cataphractus)*, Nile crocodile *(Crocodylus niloticus)*, or dwarf crocodile *(Osteolaemus tetraspis)* belly hides. Note the surface pitting (A) and dark single osteoderm buttons (B). Close views of the ventral scales are shown in figure 37.
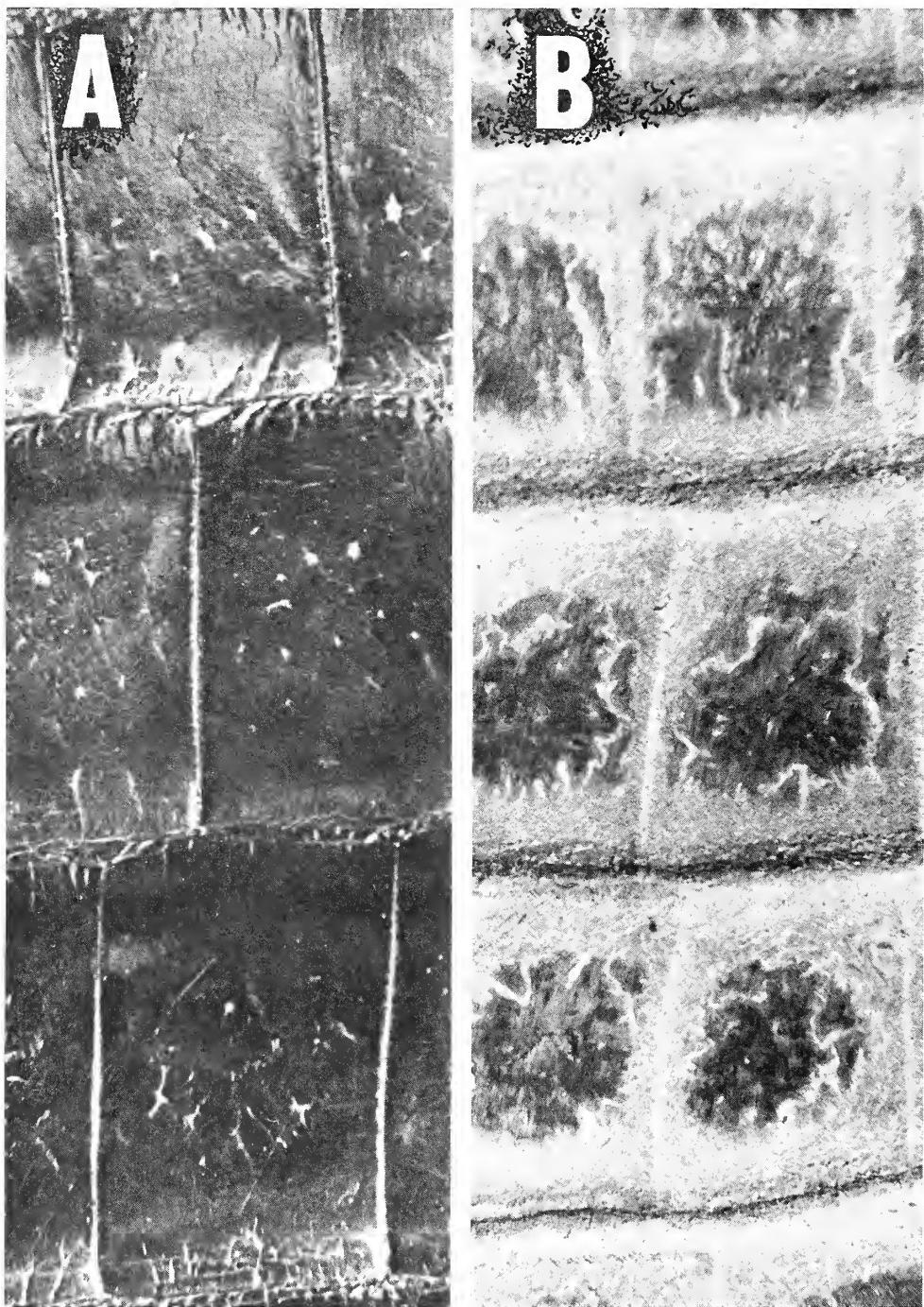
FIGURE 37. Ventral scales (A) and single osteoderm buttons (B) of finished African slender-snouted crocodile *(Crocodylus cataphractus)*, Nile crocodile *(Crocodylus niloticus)*, or dwarf crocodile *(Osteolaemus tetraspis)* hides. Because of the technique used to dye this hide, the surface pitting is white against the dark scales.

FIGURE 38. Lady's purse made from narrow South American caiman *(Caiman crocodilus)* sides. Seams where the sides are glued together are difficult to locate. Arrows indicate seams.
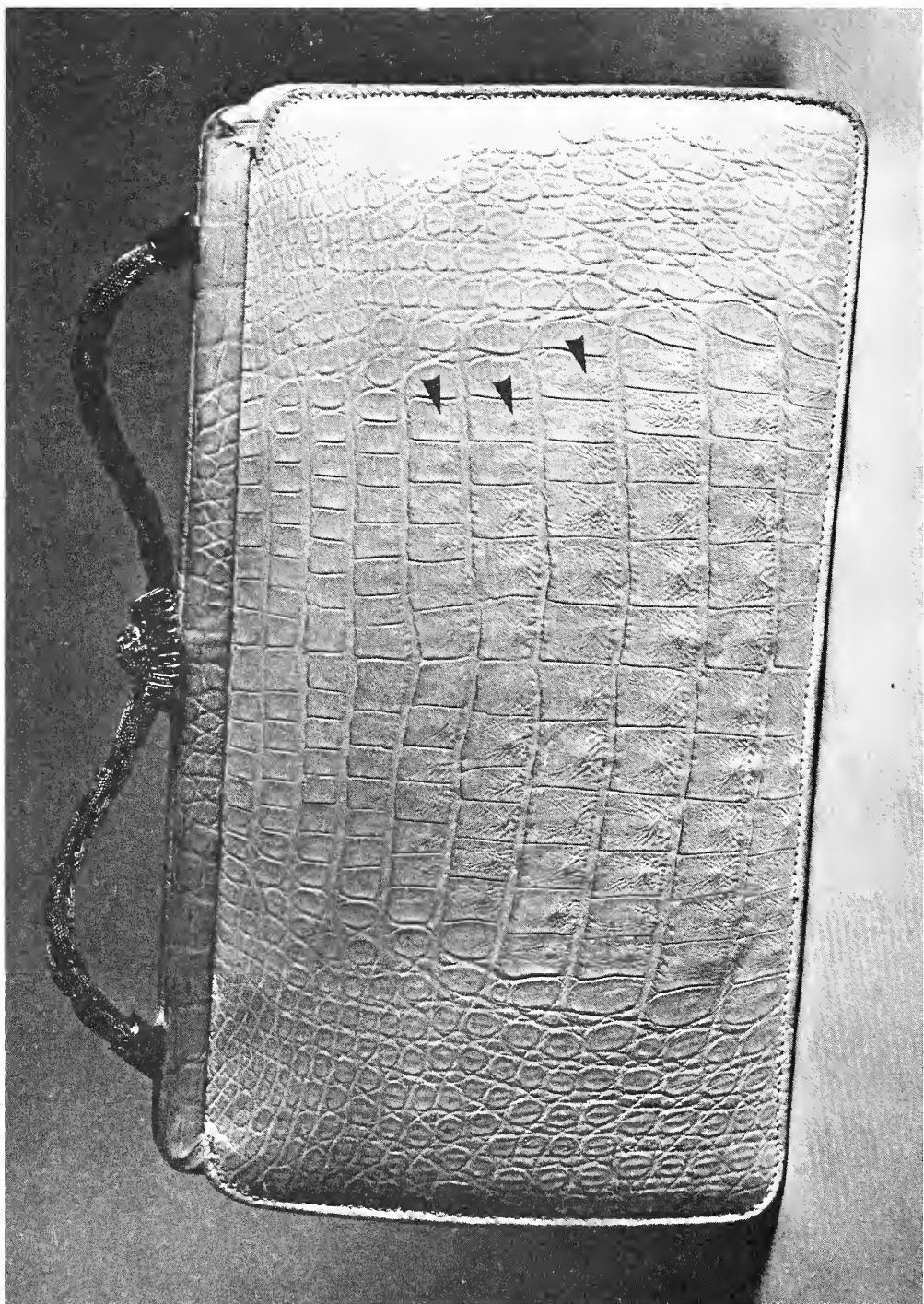
FIGURE 39. Lady's purse made from a South American caiman *(Caiman crocodilus)* belly. Note the wrinkles and surface pitting. Arrows indicate the high points of the scales. In this species the high point is just anterior to the center of the scale (the anterior end of this hide is touching the table, the posterior end is up).
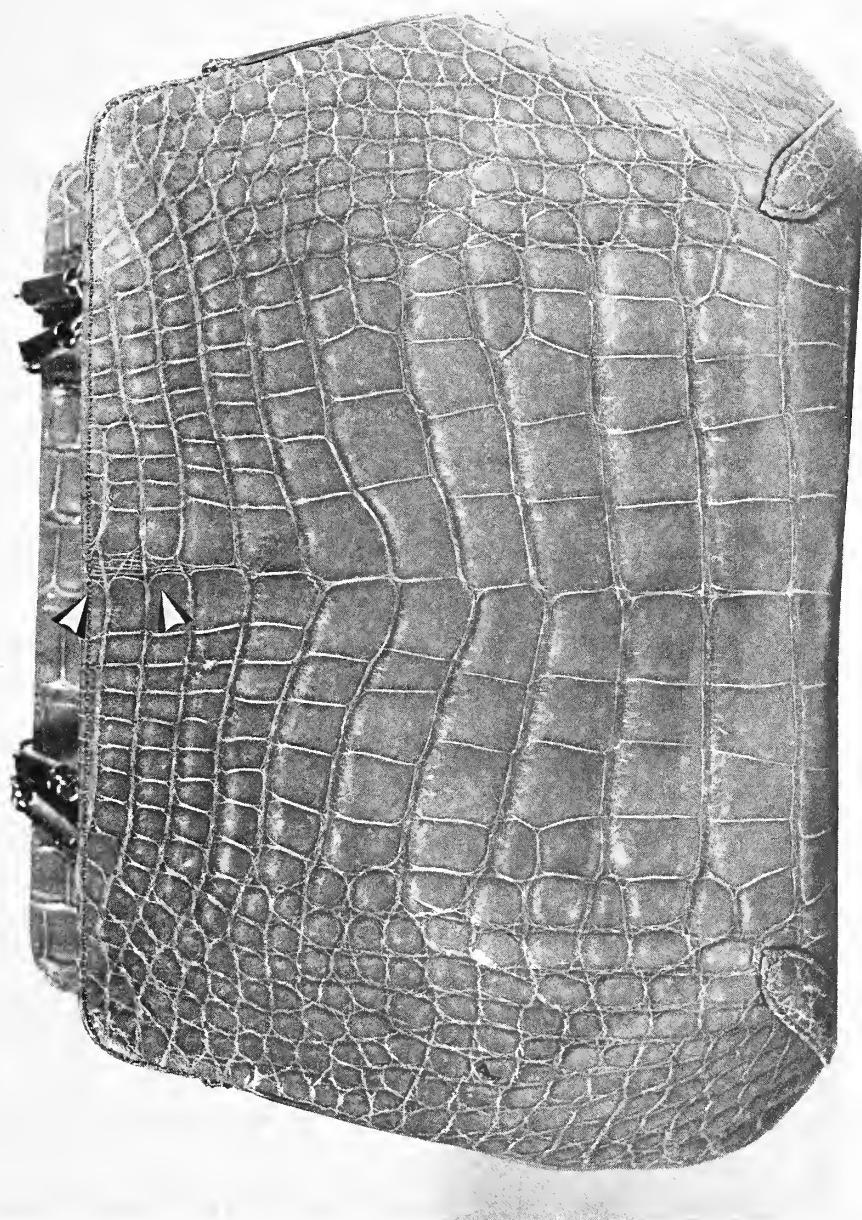
FIGURE 40. Lady's purse made from American alligator *(Alligator mississippiensis)* belly. Note the absence of both surface pitting and follicle glands. Also note the spider-web umbilicus indicated by the arrows.
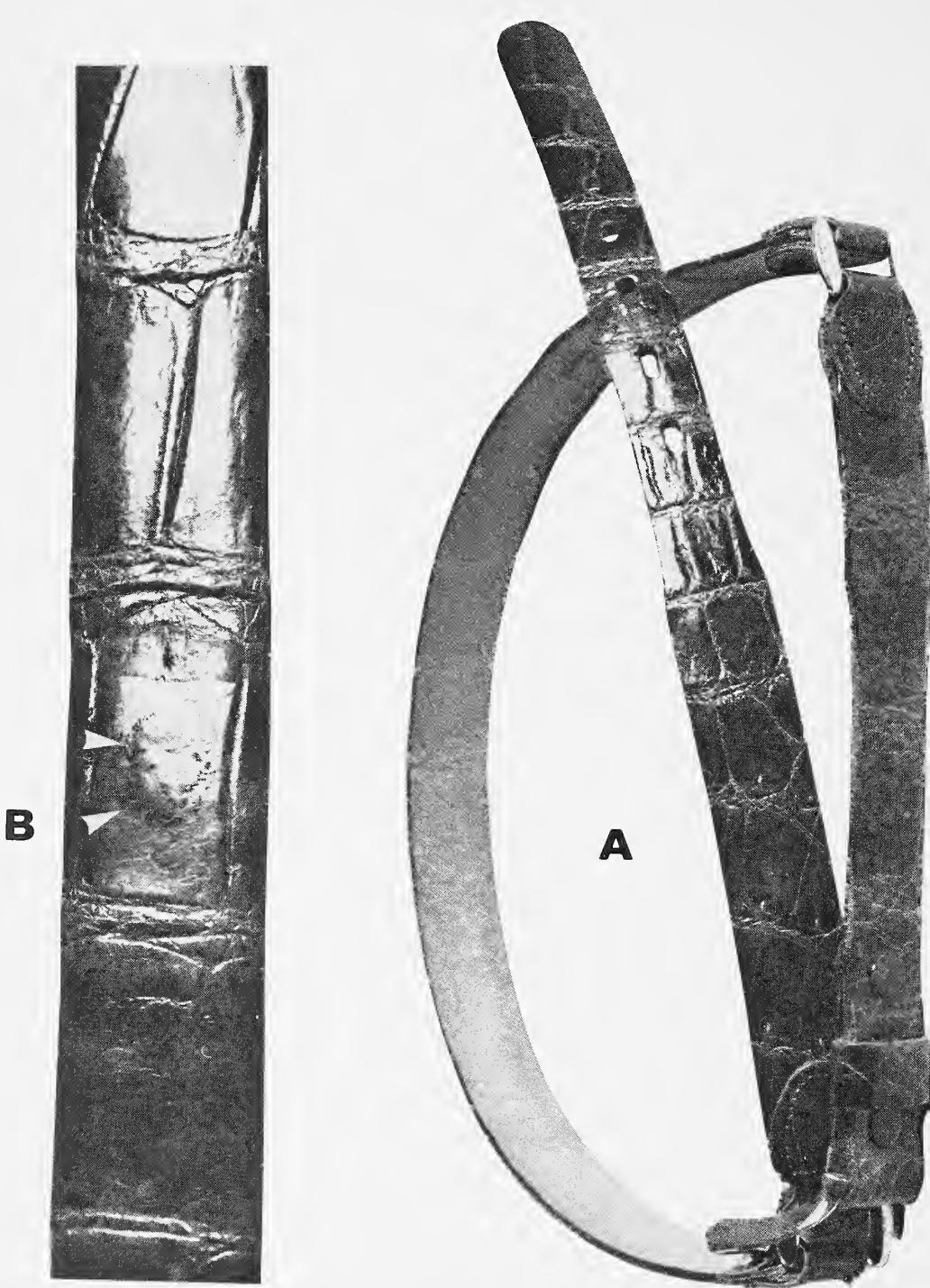
FIGURE 41. Man's belt made from either African slender-snouted crocodile *(Crocodylus cataphractus)*, Nile crocodile *(Crocodylus niloticus)*, or dwarf crocodile *(Osteolaemus tetraspis)* belly hide. Photograph B is a close view of some scales from A. Note follicle glands and also slight hump (arrows) indicating underlying single osteoderm buttons.

NEWS AND NOTES

Crocodylus intermedius Graves, A Review of the Recent Literature

(Figures 1-3)

Studies evaluating the definitive morphological characters of living crocodilians have disclosed some confusion in the recent literature on the Orinoco crocodile *Crocodylus intermedius* Graves (Mook, 1921, Bull. Amer. Mus. Nat. Hist., 44(13):165-173; Wermuth, 1953, Sonderdruck aus: Mitteilungen aus dem Zoologischen Museum in Berlin, 29(2):493-495; Wermuth and Mertens, 1961 Schildkröten, Krokodile, Brückenechsen, Veb Gustav Fischer, Jena: 359 and 361). A complete biological profile of the species is given by Medem (1958, Caldasia, 8(37):175-215) and need not be repeated here.

I thank Dr. F. Wayne King and the New York Zoological Society (= NYZS); Federico Medem; the American Museum of Natural History, New York (= AMNH), and its Herpetological Information Search System; and the Field Museum of Natural History, Chicago (= FMNH) for their assistance in making specimens and difficult-to-obtain literature available for study and for reviewing the manuscript.

DISCUSSION

Before Medem presented his collection to the Field Museum of Natural History in 1958, *Crocodylus intermedius* was poorly represented in zoological and museum collections. Those specimens which were available were supported by little or no collecting data. Consequently, one skull (AMNH 8790), bearing the data "Venezuela, South America, via the New York Zoological Society," was described in detail and figured by Mook (1921), and subsequently figured by Wermuth (1953), and Wermuth and Mertens (1961). Unfortunately, the skull was not available for re-examination until recently. Comparison of AMNH 8790 to a female *Crocodylus intermedius* collected by Medem on the Rio Ariari, Territory of Meta, Colombia (FMNH 75658); and individuals of *Crocodylus cataphractus* from K. P. Schmidt's Congo Expedition (AMNH 10075), and from Liberia, West Africa (NYZS 610716 and 610504) discloses AMNH 8790 to be an example of *Crocodylus cataphractus,* the West African slender-snouted crocodile, erroneously identified as *Crocodylus intermedius*.[1, 2]

Medem (1958:184) pointed out that Mook described and figured AMNH 8790 with nasal bones not entering the external narial opening while those *Crocodylus intermedius* he had examined from Colombia showed the nasals to enter the external narial opening. However, he did not realize Mook had incorrectly identified the specimen as *C. intermedius*.

In addition, AMNH 8790 differs from *Crocodylus intermedius* (FMNH 75658) and agrees with *Crocodylus cataphractus* (AMNH 10075), in the following aspects:

The pre-maxillary/maxillary suture extends caudad to slightly beyond the level of the first maxillary teeth in AMNH 8790, while in FMNH 75658 the suture nearly reaches the level of the third maxillary teeth.

The mandibular symphysis of AMNH 8790 and AMNH 10075 extends to the level of the eighth mandibular teeth, while in FMNH 75658 the symphysis barely reaches the level of the seventh mandibular teeth.

The palatine/maxillary suture in AMNH 8790 is triangular, anteriorly pointed at its junction with the median palatine suture, and occupies a space approximately equal to that of three adjacent maxillary teeth. FMNH 75658 has an elongated parallel-sided palatine/maxillary suture, square at its anterior face which is at right angles to the median palatine suture. Its length coincides to the space occupied by four maxillary teeth.

The ninth maxillary teeth are largest in AMNH 8790 while the tenth are the largest in FMNH 75658.

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[1] While comparing plate figures, it was noted that the skull figure for *Tomistoma schlegelii* (S. Müller) shown in Wermuth and Mertens, 1961, page 376, was duplicated in error on page 360 as the skull figure for *Crocodylus cataphractus* Cuvier.

[2] De Rochebrune, 1883 (Faune de la Senegambie, J. Durand, Imprimeur de la Societe Linneenne, Bordeau, p. 47), includes *Temsacus intermedius* Gray (= *Crocodylus intermedius* Graves) in the fauna of Senegambi (= Senegal and Gambia) although the species is unknown in Africa. The specimen he figures most closely resembles *C. intermedius*.

The conformation of AMNH 8790 is suggestive of *C. cataphractus* in the relatively high, square profile of the cranial table, the concave dorsal aspect of the snout, and the proportionately narrow frontal between the orbits. FMNH 75658 differs in having a relatively low cranial table, a slightly elevated or "swollen" snout immediately anterior to the orbits, and a frontal region which is wide in proportion to the overall length of the skull.

It should be noted that AMNH 8790 is the skull of a deformed specimen, probably resulting from confined captive conditions over a prolonged period of time during shipment. Many of the maxillary and mandibular teeth are broken or twisted in their sockets. The mandible itself is broken, perhaps during preparation or damaged in life. Portions of the anterior mandible and the pre-maxillaries are also damaged or worn away, a condition often seen in captive specimens poorly crated for shipment, in cramped quarters.

Only two "Orinoco crocodiles" appear in the New York Zoological Society's annual reports between the years 1900 and 1922. These coincide to the receipt of AMNH 8790 and another preserved juvenile specimen (AMNH 2206) bearing "Colombia, South America," data, also "via the New York Zoological Society." The latter preserved specimen is also an example of *Crocodylus cataphractus*. One of these is reported to have been secured by the zoological park from a donor recently returned from a tour aboard a merchant vessel.

One of these specimens was photographed in life while at the zoological park. The plates, misidentified as *Crocodylus intermedius,* were reproduced in subsequent literature (Ditmars, 1913, Bull. Zool. Soc., 16(58):1005; DeSola, 1933, Bull. Zool. Soc., 36(1):14, Wermuth, 1953, 29(2):493). These photographs are preserved in the NYZS photographic archives.

The identification of living crocodilians without the availability of accurate collecting data has been a problem for scientific staffs of zoological parks and museums, particularly during earlier years when the classic works of Boulenger, Cuvier, and Gray represented the only comprehensive literature on crocodilians. These publications, which stress osteological materials rather than living specimens, obviously were of little help in the identification of a rare species perhaps never seen before and seldom encountered since.

Peter Brazaitis, Department of Herpetology, New York Zoological Park, Bronx, New York 10460.
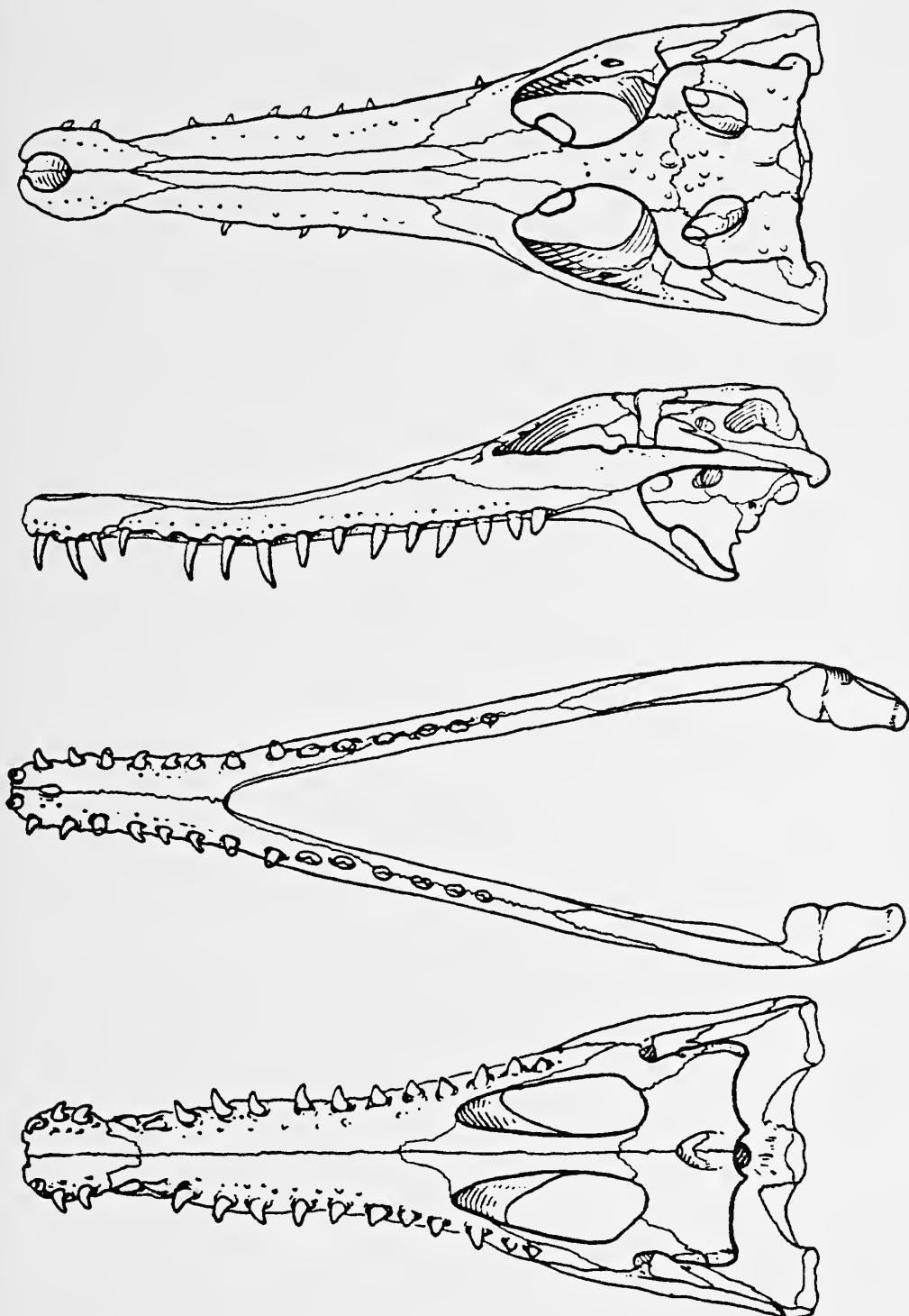
AMNH 8790

FIGURE 1. *Crocodylus cataphractus* Cuvier (AMNH 8790), misidentified and described in Mook (1921) as *Crocodylus intermedius* Graves. Figure adapted from Mook.
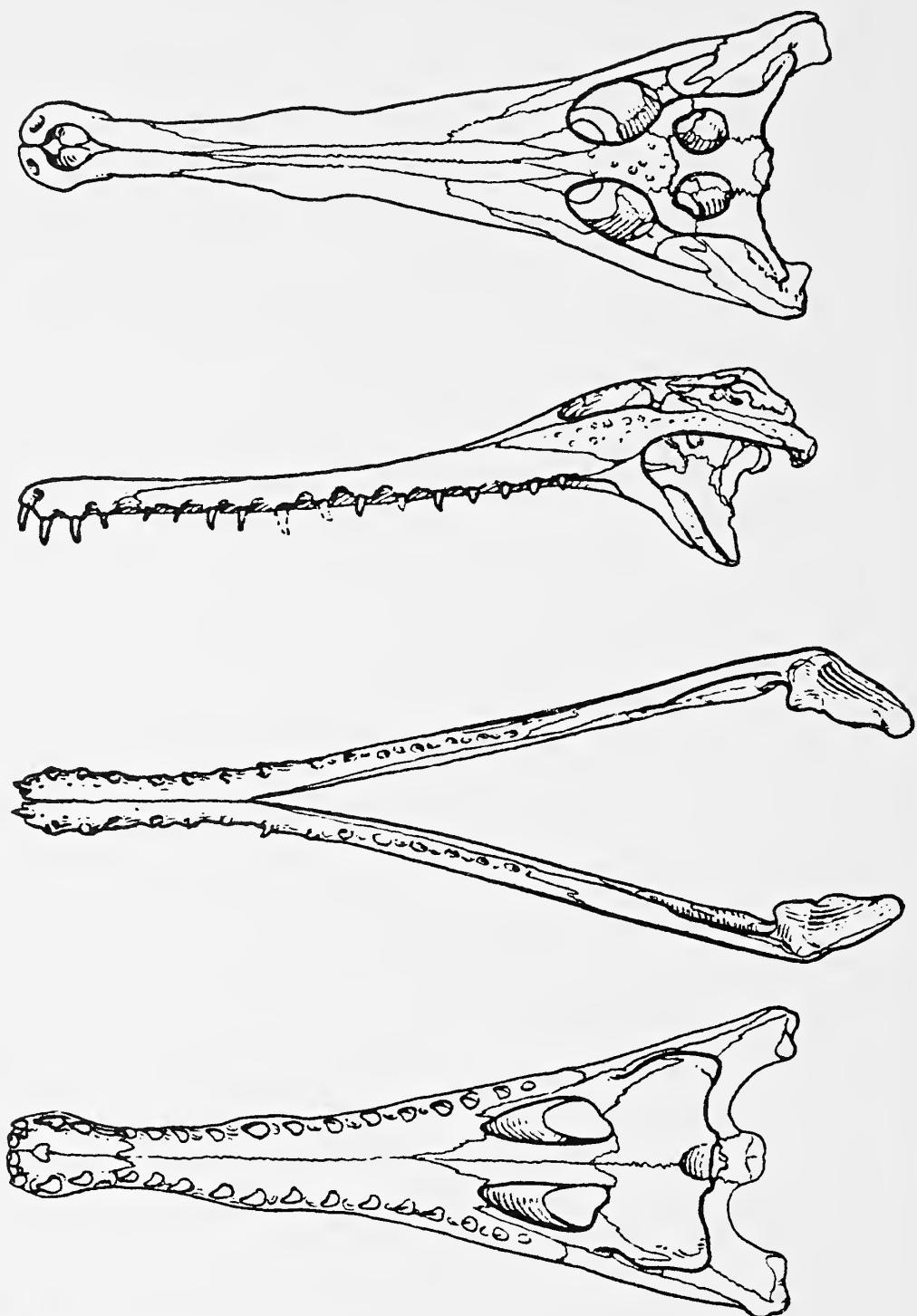
AMNH 10075

FIGURE 2. *Crocodylus cataphractus* Cuvier (AMNH 10075), described in Mook (1921). Figure adapted from Mook.
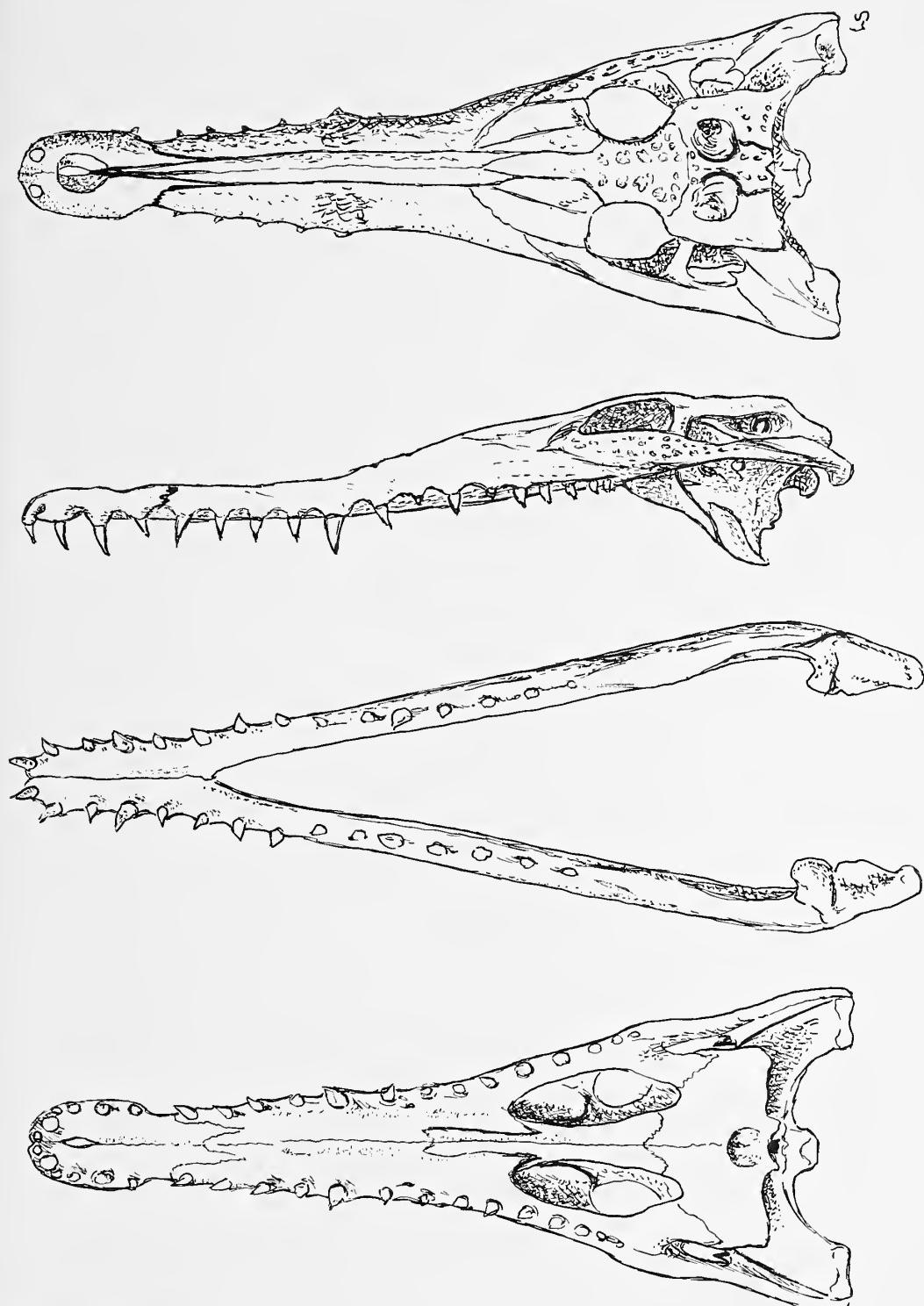
FMNH 75658

FIGURE 3. *Crocodylus intermedius* Graves (FMNH 75658), a juvenile female from Rio Ariari, Territory of Meta, Colombia, collected by Federico Medem. Illustration by Lloyd Sandford, NYZS.

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Manuscripts must conform with the Style Manual for Biological Journals (American Institute of Biological Sciences). All material must be typewritten, double-spaced, with wide margins. Papers submitted on erasable bond or mimeograph bond paper will not be accepted for publication. Papers and illustrations must be submitted in duplicate (Xerox copy acceptable), and the editors reserve the right to keep one copy of the manuscript of a published paper.

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Fifty reprints will be furnished the senior author free of charge. Additional reprints may be ordered; forms will be sent with page proofs.

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3

Inheritance of Melanophore Patterns and Sex Determination in the Montezuma Swordtail, *Xiphophorus montezumae cortezi* Rosen

Klaus D. Kallman[1]

(Figures 1-10)

The inheritance of four melanophore patterns was studied in the teleost *Xiphophorus montezumae cortezi* endemic to parts of the Rio Panuco system, Mexico. Three of them, At (atromaculatus), Cam (carbomaculatus), and Sc (spotted caudal) are composed of macromelanophores and the fourth one, Cb (caudal blot), of micromelanophores. The patterns are controlled by four loci that are not linked and not associated with sex. No abnormal sex ratios were obtained. At, Cam, and Cb are dominant, but *Sc* exhibits incomplete penetrance in the homozygous and heterozygous conditions. The penetrance of *Sc* in an inbred laboratory stock is about 88 percent; in hybrids between this stock and wild fish, the penetrance of *Sc* is only 30 percent. The frequency of the *Sc* factor in the population of the Rio Axtla has been estimated to be about 59 percent. Within the inbred stock, the expression of *Sc* may vary from a small elongate streak in the caudal fin to large melanomas that eventually destroy it. The melanoma may spread into the caudal peduncle. No fish with melanoma have been seen in preserved collections of *X. m. cortezi* or in hybrids between the inbred stock and wild fish. All the major populations studied are polymorphic for the four patterns, although there may be significant differences in their frequencies. The situation in *X. m. cortezi*, where the macromelanophore patterns are controlled by three unlinked loci, contrasts with the one present in *X. maculatus* and *X. variatus*, where the patterns are controlled by the same gene or supergene.

INTRODUCTION

THE GENUS *Xiphophorus* provides excellent material for the study of evolutionary processes at various taxonomic levels, because a variety of characters that can be analyzed genetically are present in related forms (e.g. *pigment patterns:* Anders and Klinke, 1965; Atz, 1962; Gordon, 1951; Kallman and Atz, 1966; Zander, 1962, 1969; *sex determination:* Dzwillo und Zander, 1967; Gordon, 1952; Kallman, 1965, 1968, 1970a; Kosswig and Öktay, 1955; Peters, 1964; *behavior:* Clark, Aronson and Gordon, 1954; Franck, 1964, 1970; *gonopodial traits:* Gordon and Rosen, 1951; Sengun, 1949). Nine of the 17 described species or subspecies are polymorphic for one

or more macromelanophore patterns (Kallman and Atz, 1966; Rosen and Kallman, 1969). Best studied are those of *X. maculatus*. A very large number of crosses has shown that they are controlled by sex-linked factors that are members of the macromelanophore locus. However, at least two cases among several thousand offspring are known in which two different macromelanophore genes have become linked to each other (MacIntyre, 1961; Kallman and Schreibman, 1971). There is also some evidence that a modifier is adjacent to the pigment gene that regulates its expression. Thus the macromelanophore patterns are controlled by a complex locus.

Another interesting fact that has emerged from comparative genetic studies is that identical patterns in different populations of the same species have a different genetic basis (caused by different alleles at the major pigment locus

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interacting with population-specific modifiers)
(Kallman, 1970b).

All available evidence indicates that no
macromelanophore factor is present in more
than one species, but admittedly the patterns of
species other than *maculatus* have been poorly
studied. The few crosses made with *X. variatus*
and *X. milleri* indicate that their macromelano-
phore patterns are also under the control of
sex-linked genes. The number of progeny raised
are too small, however, to determine whether
their macromelanophore factors are pseudo-
alleles also. Because the gonosomes of *macu-
latus, variatus* and *milleri* are homologous,
Kallman and Atz (1966) suggested that all three
species arose from an ancestral form (XX ♀♀—
XY ♂♂) with a sex-linked macromelanophore
locus. The two spotted patterns of *X. hellerii*,
Db^1 and Db^2, are not associated with sex; no
experiment has yet been performed that would
test whether they are alleles. The chromosome
that carries Db^1 is not homologous to the sex
chromosomes of *maculatus* (Gordon, 1958;
Kallman and Atz, 1966). Atz (1962), Kallman
and Atz (1966), and Zander (1965) pointed
out that in *X. montezumae cortezi* the two
known macromelanophore patterns, *Sc* (spotted-
caudal) and *At* (atromaculatus) were caused
by different genes that were not linked. There is
some evidence that *Sc* is located on a chromo-
some that is homologous to the sex chromosome
of *maculatus* (Breider and Mombour, 1949;
see also comment by Kallman and Atz, 1966).

During a recent field trip to the Rio Mocte-
zuma, San Luis Potosi, Mexico, some *X. m.
cortezi* were collected with macromelanophore
spotting that differed from At in consisting of
fewer but larger markings on the flank. The
present report is an account of the genetic basis
of the new pattern and also of caudal-blot, Cb,
the only known tailspot pattern for which *X. m.
cortezi* is polymorphic (Gordon, 1940; Kallman
and Atz, 1966; Rosen, 1960).

MATERIAL AND METHODS

The Montezuma swordtail, *Xiphophorus
montezumae* Jordan and Snyder, is endemic to
the Rio Panuco-Rio Tamesi drainage. Two sub-
species are recognized (Rosen, 1960). As far
as is known, *X. m. montezumae* inhabits the
headwater streams of the Rio Tamesi (Rio Frio,
Rio Sabinas, but not Rio Guayalejo) and the
northern and western tributaries (Rio Salto de
Agua, Rio Verde) of the Rio Panuco (Rosen,
1960, Darnell, 1962) while *X. m. cortezi* is
restricted to the headwaters of the Rio Mocte-
zuma and Rio Tempoal (Rio Calaboza) that
drain into the Rio Panuco from the south. With
the exception of seven fish (four from Rio
Calaboza system and three from the rather

dubious location "arroyo near Valles"), all
specimens were collected along the Pan Ameri-
can Highway between Tamazunchale, San Luis
Potosi, Mexico, and a point approximately 44 km
north of this town (figure 1). The samples come
mainly from the Rio Moctezuma, from the
Arroyo Palitla that flows into the Rio Mocte-
zuma north of Tamazunchale, from the Arroyo
Matlapa that enters the Rio Axtla from the
south, and from the Rio Axtla proper or small
streams running into it.

Fish of pedigree 1765 were collected in the
Arroyo Palitla, 13 km north of Tamazunchale
on April 21, 1965. Strain 38 has been derived
from fish that were collected in the Rio Axtla,
near the ferry crossing to Xilitla, in 1939. This
stock has been maintained in the laboratory for
21 generations. An account of this stock has
been presented by Kallman and Atz (1966).
The system of raising fish and assigning pedigree
numbers has been described previously (Gordon,
1950; Kallman, 1965). The four patterns with
which this study is concerned are:

Cb: caudal blotch, a large oval area com-
posed of micromelanophores in the proximal
portion of the caudal fin (figure 2). The Cb
pattern is also present in *X. m. montezumae* and
X. pygmaeus nigrensis (Kallman and Atz, 1966).

Sc: spotted caudal, irregular longitudinal
streaks or spots consisting of macromelano-
phores in the caudal fin (figures 3 and 4). This
gene often gives rise to melanomas in a strain
maintained in this laboratory (figure 5), but
not in natural populations.

At: atromaculatus, a large number of black
spots, composed of macromelanophores on
flank and dorsal fin. Most spots concentrated
below dorsal fin and in dorsal part of caudal
peduncle (figures 3, 4, 5, 6).

Cam (the newly discovered pattern): carbo-
maculatus (from the Latin words *carbo* for coal
and *maculatus* for spotted), relatively few but
large spots on flank. This pattern differs from
At in possessing fewer but larger markings. The
dorsal fin is only rarely spotted and then only
at the base (figures 2 and 7).

Individual spots were counted on all fish on
the left side under an x10 dissecting scope. All
fish were preserved in 10 percent formalin in
the Genetics Laboratory for future reference.
Size of fish is given in mm of standard length.

The distribution of the four patterns in
natural populations was studied by examining
the following preserved collections:
Arroyo Matlapa, San Luis Potosi, Mexico.

Gordon, Coronado, Gandy, April 14-15,
1939. UMMZ (University of Michigan, Mu-
seum of Zoology) #124374 (collected at
Comoca).
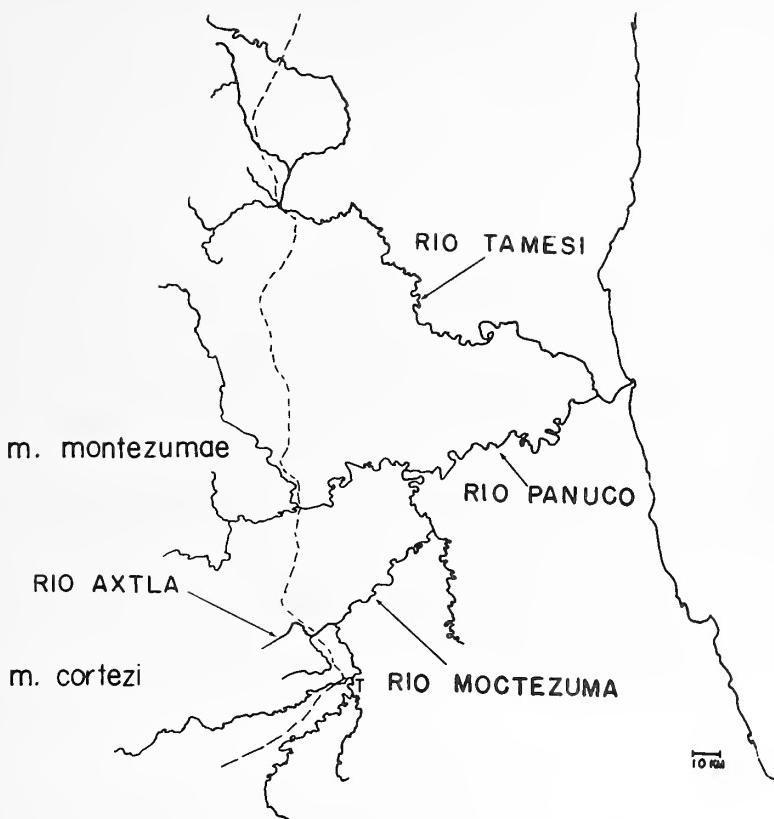
FIGURE 1. Rio Panuco-Tamesi system showing major streams. Virtually all collections of *X. montezumae cortezi* were made along a stretch of the Pan American Highway (broken line) between Rio Axtla and Tamazunchale (T).
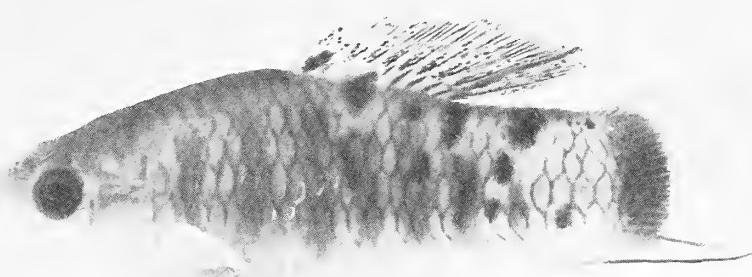
FIGURE 2. *X. montezumae cortezi*, ♂, 1765-11, 19 months after capture, 53 mm. The dark markings on the flank and the one large spot in the dorsal fin are caused by *Cam*. Note large size of the spots which often extend over several scale areas. The grayish elongate vertical bars ("parr marks") are under nervous control and are not part of Cam pattern. Such bars are found in most Montezuma swordtails. The dark crescent shaped area in the proximal part of the caudal fin is caudal blot, Cb.

FIGURE 3. *X. montezumae contezi*, ♂, strain 38, 20th laboratory generation, 12 months old, 35 mm. Heavy spotting on flank and in dorsal fin is At pattern. This pattern consists of relatively smaller spots than Cam. The irregular elongate streaks in caudal fin are caused by *Sc*, spotted-caudal.
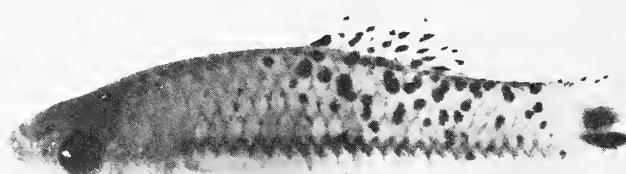
FIGURE 4. *X. montezumae cortezi*, ♀, strain 38, 19th laboratory generation, 19 months old, 44 mm. Spotting on flank and in dorsal fin is caused by At. Black mark in caudal fin is spotted caudal pattern.
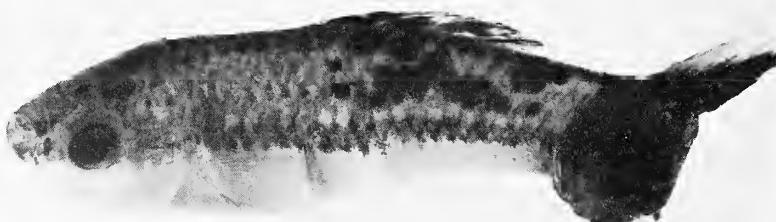
FIGURE 5. *X. montezumae cortezi*, ♀, strain 38, 21st laboratory generation, 19 months old, 42 mm. Spots on flank and in dorsal fin are caused by At. Large black area below anterior part of dorsal fin is the result of fusion of several smaller spots. Melanoma in caudal fin is caused by *Sc*.
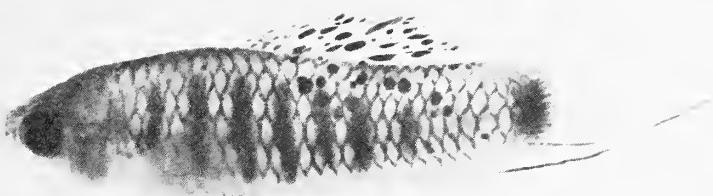
FIGURE 6. *X. montezumae cortezi*, ♂, ped. 2202, 12 months old, 34 mm. Markings on flank (17 spots) are attributed to *At* because of their small size. Caudal blot pattern is present in tail fin.

FIGURE 7. *X. montezumae cortezi*, ♂, ped. 2202, 12 months old, 43 mm. Spotting pattern on flank is attributed to *Cam*, since many of the spots (16) are of relatively large size.

FIGURE 8. *X. variatus variatus*, ♂ UMMZ #108673, 33 mm. Black area in caudal fin is caused by macromelanophores and resembles spotted-caudal pattern of *X. montezumae cortezi*.

Gordon and party, April 14, 1939. UMMZ #124341 (collected at Matlapa).

Breder, Jr., March 25, 1940. UMMZ-Station 87 of New York Aquarium Expedition.
Rio Axtla and arroyo flowing into Rio Axtla, San Luis Potosi, Mexico.

Gordon, Whetzel, Ross, April 20, 1932. UMMZ #108602 (collected at Axtla).

Gordon and Atz, January 14, 1939. UMMZ #124174 (collected at Axtla).

Breder, Jr., March 25, 1940. UMMZ-Station 84 of New York Aquarium Expedition (collected in small arroyo between Rio Axtla and Rio Moctezuma).

Robinson, Nov. 26, 1957. UMMZ #174563 (collected 1 mile before Xilitla on road from Pan Am. Hwy.).
Arroyo Palitla, San Luis Potosi, Mexico.

Gordon and party, April 13, 1939. UMMZ #124331 (collected at Palitla).

Coronado, April 2, 1940. UMMZ #186323.

McLane, Dec. 19, 1940. UMMZ #162142.

Kallman and Kallman, April 21, 1965. Genetics Lab. collection.
Rio Moctezuma, at Tamazunchale, San Luis Potosi, Mexico.

Coronado, April 1, 1940. UMMZ #186319.

Sanders, July 11, 1937. UMMZ #180036, #105682.
Rio Calaboza (Rio Tempoal) system, Veracruz, Mexico.

Creaser, Gordon and Ostos, May 5, 1930. UMMZ #108678 (collected from Rio de los Hules, 11 miles SW of Tantoyuca).

Gordon, Creaser, Ostos, May 7, 1930. UMMZ #108679 (collected in tributary of Rio Calaboza, 20 miles S of Tantoyuca).

The frequencies of the patterns have been listed in Table 4, but only fish above 25 mm of standard length have been included. Smaller fish were deemed too young; in many the patterns were poorly developed and conceivably in others the pigment genes were not expressed. Three collections (numbers 105682, 108602, 124174) consisted exclusively of small specimens and none were included in the tables. No patterns were present in the four fish from the Rio Calaboza.

The age of preserved fish cannot be determined. Generally, size increases with age, but this does not hold true for males which virtually stop growing at the time of sexual maturity. Their sizes in the above collections ranged from 20 to 51 mm of standard length. It is a well known phenomenon in *Xiphophorus* that males (even siblings raised under identical conditions) become sexually mature at different ages (Rosen, 1960; Rosen and Kallman, 1969; Peters, 1964; Zander, 1965) and this accounts in part for the large variations in size.

Results

The results of all breeding experiments are listed in Table 1 and the postulated genotypes of the parental fish are given in Table 2.

The Cb pattern is inherited as a dominant autosomal trait. One male, 1765-13, collected from a natural population (ped. 1860) and two

Table 1.

	Pedigree and phenotype of parents		
♀♀		♂♂	
1765-1	+	unknown	
1765-2	+	unknown	
1765-3	+	unknown	
38	At Sc	1765-13	Cb
38	At Sc	1765-11	Cam Cb
1797-1	+	1765-11	Cam Cb
1800-1	+	1800-12	Cb
1889b-1	Cb	1860-11	At Sc
1889b-2	Cam Cb	1962-11	+
1889b-4	+	1889a-11	At Sc
2085-1	Cb	1889a-11	At Sc
2043-1	Sc Cb	2085-12	Cam Cb
2096-1[4]	At	2085-11	Cb
2096-2[5]	Cam	2085-11	Cb
2043-2[6]	Cb	2085-13	Cam Cb
2043-3	+	2096-11	At
2096-3	+	2043-11	Sc Cb
2085-3	+	2085-14	+
2202-1	At	2214-12	Cb

[1] Age in months at which fish were scored for presence or absence of Sc.

[2] A strong Sc pattern obliterates Cb.

[3] 12 ♀♀ (2 Sc), 4 ♂♂ (3 Sc) at 8-10; 26 ♀♀ (4 Sc), 21 ♂♂ (3 Sc) at 12-15; 3 ♂♂ (1 Sc) at 19.

[4] Non-expression of *Sc* at 12 months.

[5] Non-expression of *Sc* at 17 months.

[6] Non-expression of *Sc* at 15 months.

fish bred in the laboratory, 2043-2 and 2043-11, the offspring of *Cb* parents, were homozygous for *Cb* (peds. 2258, 2277). All crosses of the type + x *Cb* yielded marked and unmarked progeny in equal frequency. When both parents were heterozygous, a ratio of 3 Cb : 1 + was obtained. A well developed Sc pattern that ex-tends over much of the central portion of the caudal fin base can totally obscure or obliterate Cb, and this accounts for the absence of this pattern in some fish of peds. 1860 and 2277.

At is inherited as a dominant autosomal trait (peds. 2043, 2096, 2202, 2222, 2270, 2471) confirming the earlier report by Kallman and

INHERITANCE OF PIGMENT PATTERNS IN *Xiphophorus montezumae cortezi.*

Pedigree and sex of offspring	At + / +	At Sc / +	At + / Cb	At Sc / Cb	Cam + / +	Cam Sc / +	Cam + / Cb	Cam Sc / Cb	+ + / +	+ Sc / +	+ + / Cb	+ Sc / Cb	Age (month)[1]
1797 ♀♀	4								15				8 (incl. Sc ♂),
1797 ♂♂	13								19	1			except 3 ♀♀, 19 ♂♂ at 12
1798 ♀♀	1								5	1			8, except At ♀
1798 ♂♂									5				at 12
1800 ♀♀									5		5		5 ♀♀, 3 ♂♂ at 6,
1800 ♂♂									9		17		5 ♀♀, 17 ♂♂ at 12, 6 ♂♂ at 16
1860 ♀♀		2[2]	27	4									see[3]
1860 ♂♂		1[2]	21	6									
1889a ♀♀	1		2										12
1889a ♂♂	1	1	1										
1889b ♀♀							2		1		1		12
1889b ♂♂							1						
1962 ♀♀									3				9-12
1962 ♂♂									3		2		
2043 ♀♀	4	2	11	4					2	1	11	4	12-20
2043 ♂♂	6	1	7	1					4		13	2	
2085 ♀♀					1		3		2		6		9 except 4 Cb, 1 +
2085 ♂♂					6		4		2		4		♀♀ and 1 + ♂ at 18
2096 ♀♀	4				1				2				8-12 except Cam
2096 ♂♂	6				1	2			2				at 17
2202 ♀♀	4	1	2	1	1	1	1	1	2	1	3	1	12
2202 ♂♂	4		5		2		1	1	2		1		
2214 ♀♀					2		6	3	3		8	2	13 except 2 Cb, 4
2214 ♂♂					2		6				5		CbCam ♂♂ at 19
2222 ♀♀	7		6						4	2	6		10
2222 ♂♂	5		6						5		11	1	
2249 ♀♀					3	2	3		7	2	4		8
2249 ♂♂					3		2	2	2	1	4		
2258 ♀♀							9				7	2	16
2258 ♂♂							4	1		1	12		
2270 ♀♀	8	1							7	1			9
2270 ♂♂	7								6				
2277 ♀♀											10	2	18 except 7 fish
2277 ♂♂										1[2]	15		(incl. 2 Sc) at 7
2319 ♀♀									8				12-18 (3 ♀♀, 3 ♂♂),
2319 ♂♂									7				21 (5 ♀♀, 4 ♂♂)
2471 ♀♀	1	2	8			1	2	1	2		4		10
2471 ♂♂			3		1		1				3		

Atz (1966). The results of ped. 2043 establish that the loci for *At* and *Cb* segregate independently. Under this assumption four classes of offspring should occur in the frequency of 3 *At Cb* : 3 *Cb* : 1 *At* : 1 + and the actual result fits this expectation rather well ($x^2 = 3.11$; $n = 3$; $0.5 > p > 0.3$). The alternate possibility that the two loci are linked is ruled out by the presence of wild-type progeny.

The Cam pattern is inherited as a dominant trait that is not associated with sex. There exists no statistically significant difference in the frequency of Cam males and females regardless from which parent the pigment factor was introduced (Cam from P_1 ♀: Cam — 12 ♀♀, 16 ♂♂; + — 21 ♀♀, 14 ♂♂, combined count of peds. 2085, 2249; Cam from P_1 ♂: Cam — 22 ♀♀, 14 ♂♂; + — 24 ♀♀, 20 ♂♂, combined count of peds. 1889 b, 2214, 2258). Crosses of the type *Cam Cb* x + + give rise to four classes of offspring indicating that the two pigment genes are not linked (ped. 1889b, 2085).

The spotted patterns, At and Cam, are controlled by two factors occupying different loci that are not linked. Crosses of an unspotted parent with one that had inherited both factors yielded offspring in the frequency of 2 At : 1 Cam : 1 + (41 At, 17 Cam, 23 +, combined count of peds. 2096, 2202, 2471). By contrast, all crosses of the type spotted x unspotted which gave rise to either At or Cam progeny, but not both, yielded marked or unmarked fish in a ratio of 1 : 1 (65 Cam, 77+, combined count of peds. 1889b, 2085, 2214, 2249, 2258; 76 At, 79+, combined count of ped. 2043, 2222, 2270). It

must be pointed out that not one At progeny was obtained from two of the crosses (Cam x + , peds. 2214, 2258) in which the wild-type parent had come from pedigrees with At offspring and, conversely no Cam fish were present in ped. 2222, although some sibs of the + parent were Cam. The results of these crosses do not support the hypothesis that the difference between the two spotted patterns are caused by modifiers.

Fish that are genotypically *At Cam* look like fish with just *At*. Two attempts were made to determine whether the more heavily pigmented fish contained both factors while the more lightly pigmented ones were merely *At*. Male 1889 a-11 was thought to possess both factors and this proved to be the case. However, female 2202-1, which looked just like any other At fish of pedigrees in which Cam did not occur, was heterozygous for both factors. Of critical importance for establishing that *At* and *Cam* are not allelic was the demonstration that a wild-type female of ped. 2096 did not carry a spotted factor unexpressed (ped. 2277). Thus the wild-type fish of ped. 2096 cannot be attributed to nonexpression of a pigment gene.

The inheritance of *Sc* is difficult to study because of its incomplete penetrance. From an inspection of Table 1 (and also of Tables III and IV of Kallman and Atz, 1966) it may appear that Sc has a polygenic basis. In a sense this is true, since obviously many modifiers are involved in bringing about the pattern. However, the series of crosses in which *Sc* was introduced into a *X. hellerii* genotype (Kallman and

TABLE 2. GENOTYPES OF PARENTS FROM MATINGS OF TABLE 1

Pedigree	♀♀								♂♂							
1797	+	+	+	+	+	+	+	+				unknown				
1798*	+	+	(Sc?)	+	+	+	+	+				unknown				
1800	+	+	+	+	+	+	+	+				unknown				
1860	At	At	Sc	Sc	+	+	+	+	+	+	+	+	+	+	Cb	Cb
1889a	At	At	Sc	Sc	+	+	+	+	+	+	+	+	Cam	+	Cb	+
1889b	+	+	+	+	+	+	+	+	+	+	+	+	Cam	+	Cb	+
1962	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Cb	+
2043	+	+	+	+	+	+	Cb	+	At	+	Sc	+	+	+	Cb	+
2085	+	+	+	+	Cam	+	Cb	+	+	+	+	+	+	+	+	+
2096	+	+	+	+	+	+	+	+	At	+	Sc	+	Cam	+	+	+
2202	+	+	+	+	+	+	Cb	+	At	+	Sc	+	Cam	+	+	+
2214	+	+	Sc	+	+	+	Cb	+	+	+	+	+	Cam	+	Cb	+
2222	At	+	Sc	+	+	+	+	+	+	+	+	+	+	+	Cb	+
2249	+	+	Sc	+	Cam	+	+	+	+	+	+	+	+	+	Cb	+
2258	+	+	Sc	+	+	+	Cb	Cb	+	+	+	+	Cam	+	Cb	+
2270*	+	+	(Sc?)	+	+	+	+	+	At	+	(Sc?)	+	+	+	+	+
2277*	+	+	(Sc?)	+	+	+	+	+	+	+	Sc	+	+	+	Cb	Cb
2319	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2471*	At	+	(Sc?)	+	Cam	+	+	+	+	+	(Sc?)	+	+	+	Cb	+

* In these pedigrees one or both parents must have been heterozygoys for *Sc*.

Atz, 1966) clearly shows that this pattern is due to a single major pigment gene. *Sc* does not appear to have been present in five related pedigrees (peds. 1800, 1889b, 1962, 2085, 2319), since none of the 94 fish exhibited the pattern.

Strain 38 is apparently homozygous for *Sc*. The pattern is present in about 77 percent of the fish (10th to 15th generation, Kallman and Atz, 1966; 16th to 21st generation, Table 3) and those that do not develop it nevertheless carry the *Sc* gene as shown by breeding experiments (Kallman and Atz, 1966). This was also the case with the female parents of peds. 2222, 2249, and 2258, and with one or both parents of peds. 2270 and 2471 (Table 1). The expression of Sc in strain 38 varies from a small elongate spot in the caudal fin to large melanomas that eventually destroy the fin (Atz, Kallman, and Nigrelli, 1963) (figure 5). This stock represents the only known example in *Xiphophorus* in which melanomas caused by macromelanophore genes occur without prior hybridization or cannot be attributed to mutation or crossing over (Kallman and Schreibman, 1971). There is some evidence that both the incidence and also the degree of Sc expression increases with age. The highest percentage of Sc individuals and tumorous fish observed was in the three sibships of the

21st generation which were maintained for longer periods of time than any other generation (Table 3). Four males did not develop the Sc pattern until they were older than 17 months. However, Sc melanomas are not only found in older fish; large tumors may be present in fish as young as seven months. The Sc melanoma invariably arises in the proximal portion of the tail fin, but may eventually encompass the entire fin and also part of the caudal peduncle. In the most severe cases, the caudal fin sloughs off. Fish with a large melanoma have a high mortality and often die several months or years sooner than their sibs without tumors. The first extant record of a Sc melanoma comes from the seventh laboratory generation. When fish of strain 38 are outcrossed to other stocks of *X. m. cortezi,* the penetrance of *Sc* becomes reduced to approximately 22 to 30 percent. This estimate is based upon two pedigrees (1860, 1889a) in which all individuals were heterozygous for *Sc* and seven others (2043, 2096, 2202, 2214, 2222, 2249, 2258) in which one-half of the fish were expected to have inherited *Sc* (Table 1).

No association of *Sc* with sex is apparent. Assuming an XX ♀♀ - XY ♂♂ mechanism for *cortezi,* fish of peds. 1860 and 1889a must have inherited Sc on the X chromosome. Conse-

TABLE 3. PIGMENT PATTERNS IN *Xiphophorus montezumae cortezi,* STRAIN 38 (16TH TO 21ST GENERATIONS; OBTAINED FROM MATINGS OF At Sc ♀♀ x At Sc ♂♂)

Generation	At		At Sc		Total		Age[1]	Tumors[2]
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂		
16A	5[3]	2	11	13	16	15	12	1
16B		1	5	4	5	5	12	0
16C	1	1	1	3	2	4	10	0
17A	2	5	5	9	7	14	9	1
17B	1		4	5	5	5	10	0
17C	3		7	5	10	5	9-12	1
18A	3	2	4	6	7	8	9-12	3
18B	2	1	3	5	5	6	11	2
18C	1	2	5	4	6	6	11, 17[4]	0
18D	1	—	4	4	5	4	11	2
19A	1	2	1	2	2	4	7	1
19B	2	—	8	7	10	7	12	2
19C	4	3	3	7	7	10	11	1
20A	—	2	2	2	2	4	8	2
20B	3	3	1	2	4	5	8	1
20C	2	1	10	12	12	13	12	5
21A	—	—	6	8	6	8	16	3
21B	3	—	14	11	17	11	17[5]	5
21C	1	—	15	11	16	11	16	15
Total[6]	35	25	109	120	144	145		

[1] Age in months at which fish were scored for presence or absence of Sc.

[2] This column does not include fish that were merely melanotic.

[3] Three of these fish may have had Sc, but spots were of small size and could conceivably be part of At.

[4] ♀ at 17 month, ♂♂ at 11 months.

[5] Four males showed no Sc at 17 months, but when rescored a year later the pattern was present in all of them.

[6] For earlier generations, see Kallman and Atz, 1966.

quently, in peds. 2043, 2096, and 2202, Sc should have been inherited in females only. However, the spotted-caudal pattern was present in both sexes. The difference in the percentage of Sc males and females is not statistically significant (because of the small number of Sc fish, the results of all three pedigrees have been combined: ♀♀: 16 Sc of 70, 22.9 percent; ♂♂: 7 Sc of 61, 11.5 percent, P=0.09).

Similarly no sex linkage of Sc can be demonstrated, if the sex determining mechanism of *cortezi* is assumed to be of the WY ♀♀ - YY ♂♂ type. Under this condition Sc males and females are expected in peds. 2043, 2096, and 2202, but no Sc females should occur in peds. 2214, 2222, 2249, and 2258. This was not the case. If the data of all four pedigrees are combined, no significant difference between the frequency of Sc males and females is observed (♀♀: 13 Sc of 88, 14.8 percent; ♂♂: 6 Sc of 73, 8.2 percent; P = 0.19).

Sc and *Cam* are not linked. The Cam pattern of ped. 2096 can be traced to 1765-11 and Sc to strain 38. It the patterns were controlled by factors on homologous chromosomes, no *Sc Cam* progeny should be present in ped. 2249. For similar reasons, the *Sc* and *At* loci cannot be linked (already reported by Kallman and Atz, 1966), since in peds. 2043 and 2202, some fish inherited both.

According to the results of ped. 2277, male 2043-11 was homozygous for *Cb* and heterozygous for *Sc*. Since 2043-11 inherited one of its *Cb* factors from 1765-11 and the other from 1765-13 while *Sc* can be traced to strain 38, the loci for *Sc* and Cb cannot be located on homologous chromosomes.

At and Cam patterns can be distinguished in sexually mature fish on the basis of number and size of spots. Cam fish have few large spots on the flank (from 1 to 16 in our experiments), and at most one or two large spots in the proximal portion of the dorsal fin. More than half of the markings of the Cam pattern, even in young individuals, are at least as large as one hexagonal unit that marks the reticulum. Often the spots extend over several scale areas. However, there is a brief period when Cam is just developing during which the size of the Cam spots is identical with those of At. By contrast, the At pattern consists of from one to several dozen small spots on the flank and in the dorsal fin. In At fish that are 12 months or older, the number of spots may become so large that adjacent spots fuse to form large irregular black patches below the dorsal fin. Such markings may become as large as those of Cam fish. This is one of the reasons why *At Cam* individuals look like those with just *At*. All pedigrees listed in Table 1 and figure 9 were scored independently

for both patterns by the author and one or two laboratory assistants with identical results in all cases. Even among offspring of crosses of the type *At Cam* x ++ (identified by a ratio 3 spotted:1 unspotted) *Cam* fish could be separated from *At* or *At Cam* progeny (peds. 2096, 2202, 2471). For example, the male of ped. 2202 with 16 spots was classified as *Cam* on the basis of large spot size and absence of spotting from the dorsal fin, while the two males with 17 spots were *At* because the size of their spots was small (figures 6 and 7). For the same reason the female of ped. 2471 with ten spots was classified as *Cam*. It must also be pointed out that the size of the spots of all fish listed as Cam in Table 1 and figure 9 was similar to that of the fish illustrated in figures 2 and 7. Observations on the etiology of the Cam pattern in ped. 2258 has shown that the small number of large spots is not due to a fusion of several smaller spots. Although when different pedigrees are compared with each other, the spot number (but never their size) of the more heavily pigmented Cam fish may overlap with the least pigmented At fish, no such overlap was observed in the three crosses in which both *At* and *Cam* segregated. It is significant that in ped. 2096 the Cam progeny had fewer spots than the At fish, although the former were scored at 17 and the latter at 12 months.

The number of spots that compose the At pattern increases with age. This is clearly illustrated by fish of ped. 1860 scored at different ages and also by a comparison of 9 to 10 months old fish of peds. 2222 and 2270 with 12 to 20 months old ones (ped. 2043). No such increase has been noted in *Cam* fish (compare peds. 2085, 2249, 8 to 9 months, with peds. 2214, 2258, 13 to 19 months).

X. m. cortezi with macromelanophore patterns have been repeatedly illustrated in the past but no distinction between At and Cam has ever been made. Only Zander (1969) has suggested that two slightly different spotting patterns may occur in *X. m. cortezi*. Photographs of fish with typical *At* can be found in Gordon (1951, Fig. 17, ♂; 1956, lower cover picture; 1957, Fig. 13, ♀ and ♂), Kosswig (1936, Abb. 3, ♀), Stöwahse and Villwock (1969, Fig. 1a, ♀), and Zander (1967, Tafel V, Abb. 18, ♀). Fish with typical *Cam* have been illustrated only by Rosen (1960, Fig. 13, ♂) and Zander (1967, Tafel V, Abb. 18, ♂). The picture of *X. m. cortezi* on page 10 of Gordon (1956) presumably refers to At as judged by the small size of the spots, but their low number and their absence from the dorsal fin makes it a somewhat doubtful identification. The phenotype of the male illustrated by Kosswig (1935, Abb. 1) is also doubtful because of the presence of only a single large spot.
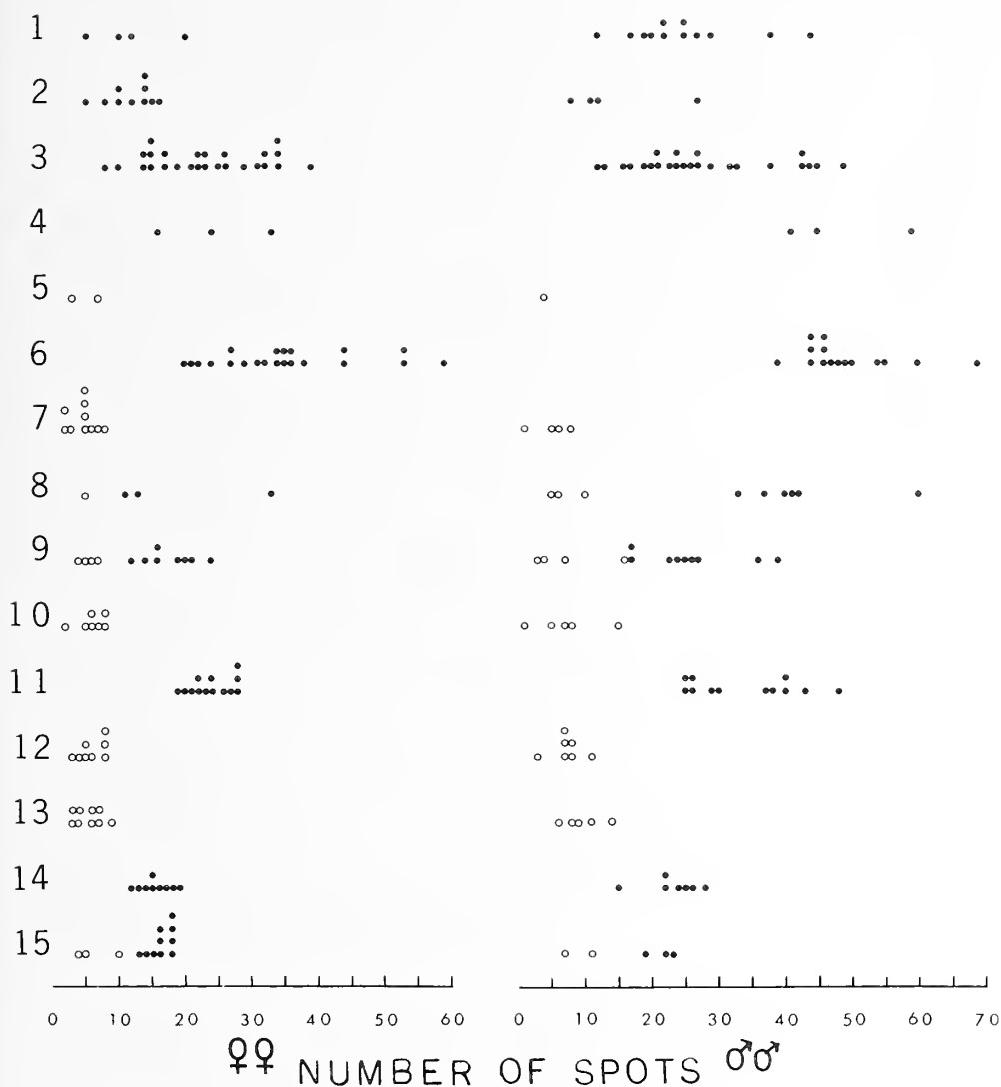
FIGURE 9. Number of spots comprising *At* and *Cam* patterns in *Xiphophorus montezumae cortezi* (laboratory broods). The ages at which the fish were scored are given in parenthesis (months). The number of spotted offspring of certain pedigrees is sometimes less than in Table 1, because some fish were used in other experiments or died (not preserved) before the spots were counted.

1) ped. 1797 (8-12)
2) ped. 1860, ♀♀ (8-10), ♂♂ (10)
3) ped. 1860, ♀♀ (12-15), ♂♂ (12-19)
4) ped. 1889a (12)
5) ped. 1889b (12)
6) ped. 2043 (12-20)
7) ped. 2085 (9)
8) ped. 2096, *At* ♀♀ (8-12), *At* ♂♂ (12), Cam ♀♀, ♂♂ (17)
9) ped. 2202 (12)
10) ped. 2214, ♀♀ (13), ♂♂ (19)
11) ped. 2222 (10)
12) ped. 2249 (8)
13) ped. 2258 (16)
14) ped. 2270 (9)
15) ped. 2471 (10)

In preserved collections from natural populations, two types of spotted patterns can be distinguished that correspond to Cam and At of laboratory reared fish (Table 3). Both patterns are present in the Rio Moctezuma, Arroyo Palitla, Arroyo Matlapa, and Rio Axtla (except UMMZ 174563), but their frequencies are not the same in the different collections. Whether these are true genetic differences between adjacent local populations or are merely due to sampling error cannot be determined. Cam fish were absent from one arroyo flowing into the Rio Axtla but made up 28 percent of the fish from the Arroyo Matlapa. The frequency of At fish in the individual samples ranged from 11 to 40 percent.

Between 25 and 32 mm of standard length, the number of spots of Cam and At patterns overlaps considerably (figure 10), but even within this range, Cam fish had usually fewer markings than those with At. Fish listed as Cam and those recorded as At with 10 or more spots have probably been correctly identified, but some of the individuals scored as At with fewer than 10 spots could conceivably have been Cam in which the pattern was just beginning to develop. Below 25 mm of standard length, only few fish can be classified unequivocally as to their pattern and, therefore, they have been omitted from Table 3 and figure 10. In larger (presumably older fish) the number of spots of At and Cam fish diverges strongly (figure 10). These data are in agreement with laboratory observations that with age the number of markings increases in At fish only.

Sc and Cb are also found in the four main locations, but Cb was absent from two collections. Cb appears to be most common in the Arroyo Palitla. The frequency of fish with Sc ranged from 6 to 43 percent.

DISCUSSION

The genetic analysis of the spotted phenotypes of *X. m. cortezi* indicates that this form has three unlinked macromelanophore loci, each possessing the wild-type (unmarked) and one pattern allele. To this author, it seems unlikely that additional patterns will be discovered in the area of the Rio Moctezuma, Rio Axtla, and Arroyo Palitla, because in the extensive collections during the last 40 years only Cam, At, and Sc were present. The four patterns occur in all of the populations sampled. Nothing is known about the populations (if any) that live upstream from Tamazunchale in the Rios Moctezuma, Amayac, and Clara, or in the Rio Calaboza system. The evolutionary events are unknown that are responsible for the difference between *maculatus, variatus,* and *milleri* in

which the numerous macromelanophore factors are all members of the same locus or supergene, and *X. m. cortezi*. Kallman and Atz (1966) have pointed out that no experiment exists that would indicate whether *At* or *Sc* or both are homologous to the macromelanophore factors of the above three species or are of independent
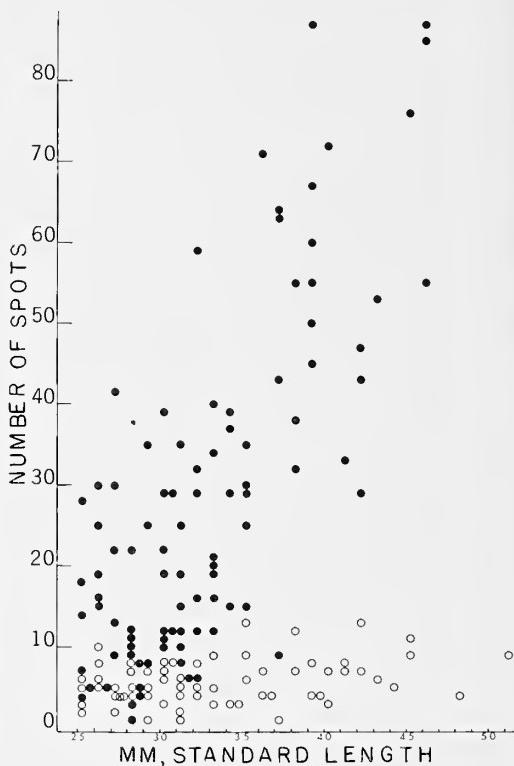
FIGURE 10. Number of spots comprising At (solid) and Cam (circle) patterns in *X. montezumae cortezi* from natural populations. There is considerable overlap in smaller fish, but above 30 mm of standard length Cam fish have fewer spots than fish with At. Larger fish with At have more spots than smaller individuals. The number of Cam spots is independent of size.

origin. To these considerations we may now add *Cam*. An alternate possibility mentioned by Kallman and Atz (1966) and Zander (1969) is that the macromelanophore factors of *cortezi* are genetically related to those of *maculatus* and were perhaps at one time members of the same complex macromelanophore locus. However, during the course of evolution they could have become separated through chromosomal re-arrangement and are now located on different chromosomes. Support for such a view is provided by the observations of Kallman and Schreibman (1971) and MacIntyre (1961) that in *maculatus* the macromelanophore factors can become separated through crossing-over. However, admittedly such crossover events within the locus are rare.

For an understanding of the evolution of the macromelanophore systems, it is also important to determine whether any of the patterns of *X. m. montezumae* are controlled by genes that are identical with *At, Cam,* or *Sc*. Preliminary results obtained in this laboratory indicate that at least some patterns of *X. m. montezumae* are sex-linked. Zander (1969) has reported that in a complicated hybrid involving *maculatus, hellerii,* and *montezumae,* a pattern of *X. m. montezumae* that he called *Sr,* was located on a chromosome that segregated from the sex chromosome of *maculatus*. If Zander's (1969) experiment can be confirmed, then four species of *Xiphophorus* are known with macromelanophore loci located on the same pair of homologous chromosomes. Since the diploid number of *Xiphophorus* is 24 (Friedman and Gordon, 1934; Lueken and Foerster, 1969), Zander's result can be taken as additional evidence for the suggestion that the macromelanophore genes of the various species can be traced to a common ancestral form.

Of particular interest is the relative high frequency of *Sc* in natural populations. This gene more than any other has to be considered as potentially deleterious, since within strain 38 it may give rise to melanomas. This strain of *X. montezumae cortezi* represents the only known example in the genus in which atypical pigment cell growths caused by a macromelanophore gene, *Sc,* occurs without prior hybridization, or cannot be attributed to mutation or crossing-over. If the penetrance of *Sc* is as low in natural populations as in the laboratory pedigrees of Table 1, the incidence of *Sc* must be considerably higher in natural populations than appears from Table 4.

Assuming a penetrance of 30 percent and no difference between homozygous and heterozygous individuals, about 83 percent of the fish from the Rio Axtla must carry *Sc*. Based upon this rather rough estimate, the frequency of this potentially injurious gene may be as high as 59 percent. Presumably a large number of modifiers are present in natural populations that keep the expression of *Sc* under control. No *X. m. cortezi* with melanotic fins or melanomas have been seen in preserved collections. This by itself cannot be taken as proof that fish with genotypes permitting the development of tumors do not occur in nature. Undoubtedly, such fish will be swiftly eliminated by predators as the melanoma develops and their swimming ability becomes impaired, and thus there will be no record. More convincing evidence for the absence or extreme rarity of such gene combinations comes from the analyses in the laboratory of broods from wild-caught females or from hybrids between strain 38 and wild stock. No melanomas were seen in such offspring (the data from Table 1 of this investigation and from Table 3 of Kallman and Atz, 1966). The presence of tumors in strain 38 must be a product of selection in the laboratory. Although no deliberate attempt has ever been made to increase the incidence of melanomas, it has consistently been the practice of our laboratory assistants to choose as parents for the following generation fish with well developed Sc patterns at the age of 9 to 10 months.

The only other pattern similar to Sc of *X. montezumae cortezi* is known from the populations of *X. variatus variatus* inhabiting the Rio Cazones system (Rosen, 1960). Morphologically this pattern (figure 8) is indistinguishable from that of *cortezi*. Based upon two collections (Gordon, Atz, Whetzel, station 45, March 31, 1948; Arroyo Mariandrea at Mariandrea, Puebla, Mexico, about 10 miles W of Poza Rica on road to Apapantilla; Gordon, Creaser, Ostos, UMMZ #108673, May 11, 1930, unnamed arroyo, near Agua Fria, 12 miles S of Miahuapan), this pattern is present in approximately 10 percent of the population. No Sc-like pattern was seen in any other *X. variatus* collection (for complete list, see Rosen, 1960). According to Atz (1962) and Zander (1969), the expression of *Sc* of *X. m. cortezi* is suppressed when introduced into *X. v. variatus* [*variatus* from Rio Axtla (Atz) and of unknown geographic origin (Zander)]. The spotted-caudal patterns of *variatus* and *cortezi,* therefore, may be caused by different genes. The crucial test, however, can only be provided by introducing the factors responsible for the patterns in the two species on to common genetic backgrounds.

Closely related to the problem of the polymorphic macromelanophore (and xanthoerythrophore) patterns is the one concerned with the evolution of mechanisms for sex determination. As was pointed out in the introduction, the gonosomes of three species, *X. variatus, X. milleri,* and *X. maculatus,* are

homologous. The first two have a XX ♀♀ — XY ♂♂ mechanism, while in natural populations of *X. maculatus,* three types of females, XX, WX, WY, and two types of males, XY and YY, may occur (Kallman, 1965 and 1970a). Although crossing over between the Y and W chromosomes has been observed repeatedly in the laboratory, no marked W chromosomes have yet been found in wild populations (Kallman, 1970a and unpublished). Macromelanophore and other pigment factors, however, are present on the X. Zander (1968) has recently discovered two pigment factors, *Vfl* and *Fl,* in two populations of *X. pygmaeus nigrensis* that have only minimal phenotypic effects within their own species, but manifest themselves as strikingly

TABLE 4. MELANOPHORE PATTERNS IN WILD POPULATIONS OF *Xiphophorus montezumae cortezi*
Patterns in preserved fish

Location		+	Cam	At	Sc	Cb	At Sc	Cam Sc	Cb Sc	Cb At	Cam Cb	Cam Sc	Cb At Sc	%
Axtla														
Sta. 84 N.Y.A.	♀♀	19	3	16		2				1				
	♂♂	7	3	6	5		2	1		2				Cam 5
174563	♀♀	9		8	14		4							At 36
	♂♂	13		8	6		2							Sc 26
														Cb 4
Matlapa														
124374	♀♀	14	13	7		2	1				2			
	♂♂	23	9	2	6	3					1	1		Cam 28
Sta. 87 N.Y.A.	♀♀	8	3		1	2								At 17
	♂♂	8	6	3	1	1		3		1				Sc 14
124341	♀♀	7	3	6	2									Cb 8
	♂♂	8	2	4	3		3	1						
Palitla														
124331*	♀♀													
	♂♂	4		6	3	1	1	2		2				Cam 14
16242	♀♀	13	2	5	3	4	3			1	1			At 29
	♂♂	11	2	11	9	2	2	1		3	1	1	1	Sc 23
186323	♀♀	1												Cb 20
	♂♂	8	3	2	1	1	2		2	1				
K & K, 1965	♀♀	5		2		1		1		1				
	♂♂	4	1			1				2				
Moctezuma														
180036	♀♀			1										
	♂♂		1	1										Cam 20
186319	♀♀	4	1	1										At 33
	♂♂		1	1	2	1	1							Sc 20
														Cb 7

* Not included in percentage calculations, since this is obviously a selected sample. This collection consisted of 319 specimens, but only 19 mature males are extant.

red body patterns after introgression into *X. maculatus*. Most significant is that *Vfl* is located on a chromosome that is homologous to the Y chromosome of *maculatus* and can replace it functionally. Although no numbers were reported, Zander's experiment suggests that in one of his *nigrensis* stock, sex-determination is by the XX ♀♀ —XY ♂♂ mechanism, since all males but none of the females inherited *Fl*. Because of the homology of the sex chromosomes of *maculatus, variatus,* and *milleri,* it is unlikely that the same pair of undifferentiated chromosomes had evolved independently into gonosomes three times (or four times if *X. pygmaeus nigrensis* is included). Presumably, the ancestral species already had a XX-XY mechanism with a macro-melanophore locus. The W chromosome of *maculatus* may be a recent innovation (Kallman, 1970a).

The sex determining system of *X. hellerii* is not well understood because of widely fluctuating sex ratios (Kallman and Atz, 1966; Peters, 1964). Several authors are of the opinion that sex determination in this genus has evolved from an ancestral condition in which sex determination was achieved polygenically by the segregation of many M or F factors scattered over many chromosomes to one with well defined gonosomes (Anders and Anders, 1963; Dzwillo and Zander, 1967; Gordon, 1952; Kosswig, 1964; Peters, 1964). According to these investigators, the original condition, or one similar to it, is still present today in *X. hellerii*. An alternate possibility has been suggested by Kallman (1965, 1968) and Kallman and Atz (1966).

It is therefore of considerable interest that an early experiment (but unfortunately based upon hybrids of unknown origin with the identity of some of the relevant pigment genes in doubt) by Breider and Mombour (1949) suggested that the chromosome of *cortezi* that carries *Sc* may be homologous to the sex chromosomes of *maculatus*. The crosses listed in Table 1, limited as they are because of the low penetrance of *Sc,* do not indicate sex linkage for *Sc*. This does not preclude the possibility, however, that the chromosome on which *Sc* is located is homologous to the gonosomes of *maculatus* and the other species, but that within *X. m. cortezi* this chromosome plays no role in sex determination.

The data published previously by Kallman and Atz (1966) did not provide any evidence for or against the presence of a XX ♀♀—XY ♂♂ or any other sex chromosome mechanism in *X. m. cortezi*. Of the forty pedigrees reared in the Genetics Laboratory only two showed a significant deviation from unity. Similarly, Kosswig (1959) did not find any significant preponderance of one or the other sex. Only Zander (1965) reported some crosses in which virtually all off-

spring differentiated into females (e.g. one male mated to three females sired 105 offspring, all but two females; a second male mated to the same three females gave rise to 183 females and one male). According to his interpretation, *X. m. cortezi* possesses an XX ♀♀—XY ♂♂ sex determining mechanism that can easily be upset by autosomal factors. Males that sire predominantly female broods have two X chromosomes and a certain number of autosomal male determining factors that override the action of the sex chromosomes. The male determining potency of the Y chromosome of *cortezi* is less than that of the Y of *maculatus* (Zander, 1965).

Zander pointed out that *X. m. cortezi* may have reached a stage in the evolution of sex-determining mechanisms that is intermediate betwen a polygenic (original) one and one in which sex is determined strictly by gonosomes. In the *Sc* males of his experiments, sex determination was thought to have been by sex chromosomes (XY ♂♂), but in the males without *Sc* to have proceeded polygenically (XX). However, the significance of these relationships was not clear (Zander, 1965).

Females with the exceptional sex genotype XY and males that are XX are known from *X. maculatus* and other species (see summary by Kallman, 1968), and there is no reason to assume that this condition cannot occur in *cortezi* as well, if indeed it has a XX-XY system. The abnormal sex ratio of 288 ♀♀ : 3 ♂♂ is certainly strong evidence that the male parents of these broods possessed a sex genotype more characteristic for females, but, curiously, when these males were mated to other females normal sex ratios were obtained.

Because the sex chromosomes of *X. maculatus* carry dominant sex-linked marker genes, the recognition of genetic sex reversals (i.e. XX ♂♂ and XY ♀♀) presents no problem in this species. This is not the case for *X. m. cortezi* and many other species. In this context it must be pointed out that significant deviations from an expected 1:1 sex ratio cannot *a priori* be attributed to genetic sex reversals as has been done by Schröder for *Poecilia* (1964) and Zander for *X. m. cortezi*. Other independently arrived corroborative evidence, e.g. sex-linked marker genes or chromosome analysis, is needed in each case. Kallman (1965) working with *X. maculatus* found that the deviation from the expected sex ratio (ped. 1485, 23 XX ♀♀, 44 XY ♂♂, Table 15) was due to the deficiency of one class and not due to genetic sex reversals.

To me the conclusion is not justified that XX males are not exceptional for *cortezi* and occur rather frequently (XX-Ausnahme-♂♂ sind für *cortezi* keine Besonderheit und treten relativ häufig auf), since nowhere did Zander (1965)

indicate just how frequently broods with a significant excess of females are obtained. Nothing was reported about the sex ratios or breeding performance of his stock. The data of Kallman and Atz (1966) and Kosswig (1959) do not show that unbalanced sex ratios are of common occurrence in *cortezi*.

Zander's interpretation was in part based upon the analysis of sex ratios of hybrids between *cortezi* and *maculatus* and *cortezi* and *variatus*. Sex determination in species hybrids of *Xiphophorus* while sometimes proceeding normally, as e.g. in *maculatus* x *pygmaeus* hybrids (Zander, 1968), is just as often contrary as to what one would expect from the sex chromosome genotype of the hybrids (provided one uses species with marked gonosomes which is not the case with *cortezi*). Kallman and Atz (1966) found that in *maculatus* x *milleri* hybrids, sex determination was largely governed by the sex chromosomes (XX ♀♀ XY ♂♂) provided the *maculatus* parent came from the Gp stock. But when the *maculatus* parent came from stock Hp-2, more than half of the XX offspring differentiated into functional males, which upon breeding with XX females of either species yielded progeny (large numbers) with a sex ratio that approached unity, mimicking XX ♀♀ x XY ♂♂ crosses. Other examples of atypical cases of sex determination are provided by Zander's (1968) *milleri-pygmaeus* crosses and by the *hellerii* x *maculatus* hybrids (summary in Table 26, Kallman, 1965). Even Zander (1965) admits that some of the results of his hybrid crosses fit into his scheme of sex determination for *cortezi* only with difficulty or not at all.

The crosses listed in this paper do not help to clarify the sex chromosome mechanism of *X. m. cortezi*, because no sex-linked marker genes have been found. However, these results, as our earlier ones, clearly show that males (XX ?) that sire predominantly female broods are not of common occurrence in *cortezi*. Some of the crosses (Table 1) were among fish that represent a pure line from Arroyo Palitla while others are hybrids between Arroyo Palitla and strain 38 (Rio Axtla). Sex ratios for strain 38 are listed in Table 3. Of all pedigrees, only two (Table 1) showed a sex ratio that deviated significantly (at the 0.05 level) from unity (ped. 1800: 10 ♀♀ —26 ♂♂, 0.01 < P < 0.02; ped. 2471: 20 ♀♀ —8 ♂♂, 0.02 < P < 0.05).

SUMMARY

The inheritance of four melanophore patterns was studied in the teleost *Xiphophorus montezumae cortezi*. Three of them, At (atromaculatus), Cam (carbomaculatus), and Sc (spotted caudal) are composed of macromelanophores, and the fourth one, Cb (caudal blot), of micromelanophores. The patterns are controlled by four loci that are not linked and not associated with sex. No abnormal sex ratios were obtained. At, Cam, and Cb are dominant, but *Sc* exhibits incomplete penetrance. In approximately 22 percent of the *Sc* fish of an inbred laboratory stock, the gene does not manifest itself; in hybrids between this stock and wild fish, the penetrance of Sc is only 30 percent. The frequency of the *Sc* factor in the population of the Rio Axtla has been estimated to be about 59 percent. Within the inbred stock, the expression of Sc may vary from a small elongate streak in the caudal fin to large melanomas that eventually destroy the fin. The melanoma may spread into the caudal peduncle. No fish with melanoma have been seen in preserved collections of *X. m. cortezi* or in hybrids between the inbred stock and wild fish. All the major populations studied are polymorphic for the four patterns, although there may be significant differences in their frequencies. The situation in *X. m. cortezi* where the macromelanophore patterns are controlled by three unlinked loci contrasts with the one present in *X. maculatus* and *X. variatus* where the patterns are controlled by the same gene or supergene.

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4

Eastern Pacific Expeditions of the New York Zoological Society. Stomatopod Crustacea.

Raymond B. Manning[1]

(Figures 1-3)

Nineteen species of stomatopods were taken by the Eastern Pacific Expeditions of the New York Zoological Society in 1936 and 1937-38. *Lysiosquilla desaussurei* (Stimpson) is redescribed and *Gonodactylus zacae* is newly described. The collections include the second record for each of ten species.

Introduction

In 1936 and again in 1937-38, the New York Zoological Society sponsored two expeditions to the tropical eastern Pacific region under the direction of William Beebe. During the first of these, the Templeton Crocker Expedition (1936), collections were made on the west coast of Baja California, the southern portions of the Gulf of California, as well as off Clarion Island and the Revillagigedo Islands. During the second expedition, the Eastern Pacific *Zaca* Expedition (1937-1938), collections were made at several localities between southern México and Gorgona Island, Colombia. Stations for both expeditions are shown in Figure 1.

The stomatopod crustaceans collected during these expeditions were loaned to me for study by Dorothy E. Bliss, American Museum of Natural History. Except for one lot of paratypes of a new *Gonodactylus* described in the report and one female *Lysiosquilla desaussurei* which have been deposited in the collection of the Division of Crustacea, National Museum of Natural History, Smithsonian Institution (USNM), all of the specimens are in the collection of the American Museum of Natural History.

I am indebted to Dorothy Bliss for allowing me to work with this interesting collection and to Horton H. Hobbs, Jr., for taking his time to comment on the manuscript. Figures 2 and 3 were prepared by my wife Lilly.

Although relatively little information on the stomatopods of the eastern Pacific region has been published since the review of the eastern Pacific species by Waldo L. Schmitt in 1940, there have been several name changes at the generic level which affect the nomenclature of the eastern Pacific species. In addition, several new families have been recognized within the stomatopods. For these reasons, keys to the eastern Pacific species of each of three families, Gonodactylidae, Lysiosquillidae, and Squillidae, are presented below.

The collections reported here were found to include a new species, *Gonodactylus zacae*; a species not recorded since its original description in 1857, *Lysiosquilla desaussurei* (Stimpson); and a series of *Gonodactylus festae lalibertadensis* which indicates it should be accorded specific status. In addition, the collections include only the second record for ten species, seven of which were described by Schmitt (1940). Overall, these collections add greatly to our knowledge of the species composition of the eastern Pacific stomatopods and add information on their geographic ranges; significant range extensions are reported for several species.

Unfortunately, relatively little ecological information was included in the locality data. Such information, as well as observations on color in life of stomatopods, will be increasingly needed in future systematic studies.

Synonymies are generally restricted to the original references, to an earlier review of the eastern Pacific stomatopods by Waldo L. Schmitt (1940), and subsequent papers; citations of earlier papers can be found in Schmitt's report.

Terms and measurements used herein have been discussed in more detail elsewhere (Manning, 1969). All measurements are in millimeters (mm). Total length (TL) is measured from the anterior margin of the rostral plate to the apices of the submedian teeth of the telson; carapace length (CL) is measured on the mid-

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TABLE 1. SPECIES OF STOMATOPODA COLLECTED AT EACH OF THE STATIONS OF THE EASTERN PACIFIC
EXPEDITIONS, NEW YORK ZOOLOGICAL SOCIETY, 1936, 1937-38.

MEXICO:

E of Cedros Island, Lower California	126 D-11	....	*Meiosquilla polita*
Arena Bank, Gulf of California	136 D-18	....	*Hemisquilla ensigera californiensis*
Arena Bank, Gulf of California	136 D-30	....	*Pseudosquillopsis marmorata*
			Gonodactylus zacae
Santa Inez Bay, Gulf of California	141 D-3	...:........................	*Gonodactylus zacae*
Santa Inez Bay, Gulf of California	141 D-4	....	*Squilla tiburonensis*
	143 D-5	....	*Squilla tiburonensis*
Mazatlán	155 D-1	....	*Squilla panamensis*
Chamela Bay	shore	....	*Meiosquilla oculinova*
Tenacatita Bay	183 D-2	sandy mud	*Squilla hancocki*
	183 D-3	muddy sand	*Squilla panamensis*
Sihuatenejo Bay	shore	coral	*Pseudosquilla adiastalta*
			Gonodactylus stanschi
Port Guatulco	195 D-3-8, 14	rocks, sand, algae, cr. shell	*Gonodactylus zacae*
Port Guatulco	195 D-15	coral	*Pseudosquilla adiastalta*
			Gonodactylus stanschi
Tangola-Tangola Bay	196 light	surface	*Lysiosquilla desaussurei*
Tangola-Tangola Bay	196 D-16-18	mud	*Meiosquilla swetti*
			Squilla hancocki
			Squilla parva
			Eurysquilla veleronis

EL SALVADOR:

La Libertad	198 D-1, 2	mud	*Squilla parva*
Gulf of Fonseca	199 D-1, 12	sand, mud cr. shell	*Squilla aculeata aculeata* *Squilla parva*
Gulf of Fonseca	shore	....	*Gonodactylus festae*

NICARAGUA:

Gulf of Fonseca	199 D-4	mud	*Squilla aculeata aculeata*
Corinto	200 D-20	mangrove leaves	*Meiosquilla swetti*

COSTA RICA:

Port Parker	203 D-4, 7, 9	gravel, shells, algae, coral	*Gonodactylus zacae*
Port Parker	shore	coral	*Pseudosquilla adiastalta*
			Gonodactylus zacae
			Gonodactylus festae
			Gonodactylus bahiahondensis
Port Culebra	206 D-3	sandy mud	*Meiosquilla dawsoni*
			Squilla parva
Port Culebra	shore	coral	*Gonodactylus lalibertadensis*
			Gonodactylus bahiahondensis
Piedra Blanca Bay	208 L-1	surface	*Lysiosquilla desaussurei*
Piedra Blanca Bay	shore	tidepool	*Gonodactylus festae*
Jasper Island	shore	coral	*Pseudosquilla adiastalta*
			Gonodactylus bahiahondensis
off Ballenas Bay, Gulf of Nicoya	213 D-11, 13-15, 17	mud	*Squilla panamensis*
Uvita Bay	shore	coral	*Gonodactylus lalibertadensis*
			Gonodactylus bahiahondensis

PANAMA:

Gulf of Chiriqui	221 D-1, 4	sandy mud	*Hemisquilla ensigera californiensis*
			Squilla panamensis
Bahia Honda	222 D-1, 5	rocks, dead coral, mud shells, leaves	*Gonodactylus zacae*
			Squilla parva
Bahia Honda	shore	under stones	*Gonodactylus bahiahondensis*

FIGURE 1. Shore collecting stations of the Eastern Pacific Expeditions of the New York Zoological Society.

line and does not include the rostral plate. Species marked with an asterisk (*) in the keys are also reported in the text of this report.

Species taken at each of the collecting stations are listed in Table 1. Station data are presented in the species accounts; additional information may be found in Beebe (1937, 1938), who gave general summaries of the expeditions together with data for each of the stations.

SYSTEMATIC ACCOUNT
ORDER STOMATOPODA LATREILLE

Key to Families of Stomatopoda from the Eastern Pacific Region

1. Telson lacking sharp median carina; propodi of posterior three maxillipeds broad, beaded or ribbed ventrally. . Lysiosquillidae

1'. Telson with sharp median carina; propodi of posterior three maxillipeds slender, not beaded or ribbed ventrally. 2

2. More than four intermediate marginal denticles present on telson. . . . . . . .Squillidae

2'. No more than two intermediate marginal denticles present on telson .Gonodactylidae

FAMILY LYSIOSQUILLIDAE

Key to Genera and Species of Lysiosquillidae from the Eastern Pacific Region

1. Distal segment of endopod of anterior two walking legs elongate; proximal portion of outer margin of uropodal endopod at most angled inward, not folded. 2

1'. Distal segment of endopod of anterior two walking legs ovate or subcircular; proximal portion of outer margin of uropodal endopod with strong fold. 7

2. Dactylus of raptorial claw inflated basally; propodus of claw pectinate proximally only; rostral plate rounded or subrectangular; *Coronida* Brooks; *C. bradyi* (A. Milne-Edwards, 1869); Schmitt, 1940.

2'. Dactylus of raptorial claw not inflated basally; propodus of claw fully pectinate; rostral plate cordiform or triangular. . . .3

3. Median dorsal surface of telson with at most a low, triangular boss; marginal teeth of telson usually fused, movable submedian teeth absent (in American species); *Lysiosquilla* Dana 4

3'. Median dorsal surface of telson with raised median projection, lobed or spined posteriorly; movable submedian marginal teeth of telson always present, remainder of teeth and denticles distinct, not fused; *Heterosquilla* Manning 5

4. Dorsal surface of telson and sixth abdominal somite smooth, not tuberculate. . . . .*L. maculata* (Fabricius, 1793); Schmitt, 1940.

4'. Dorsal surface of sixth abdominal somite and telson rough, tuberculate. . . . .*L. desaussurei* (Stimpson, 1857).

5. Telson with two intermediate marginal denticles (subgenus *Heterosquilla*) . . . .*H. polydactyla* (von Martens, 1881); Schmitt, 1940; Bahamonde, 1968; Manning, 1969.

5'. Telson with four intermediate marginal denticles (subgenus *Heterosquilloides*) 6

6. Sixth abdominal somite unarmed dorsally; raptorial claw with four teeth. . . . .*H. mccullochae* (Schmitt, 1940); Manning, 1969.

6'. Sixth abdominal somite with three pairs of spines; raptorial claw with eight teeth. . . . .*H. insolita* (Manning, 1963); Manning, 1969.

7. Dorsal surface of telson with fan-shaped series of five or more spines (posterior margin of dorsal surface of telson not produced into false eave overhanging true marginal armature); *Acanthosquilla* Manning . . . . . *A. digueti* (Coutière, 1905); Schmitt, 1940.

7'. Dorsal surface of telson unarmed or with at most a single median projection (posterior margin of dorsal surface of telson produced into a false eave overhanging true marginal armature); *Nannosquilla* Manning 8

8. Spines of basal prolongation of uropod subequal in length or outer longer than inner 9

8'. Inner spine of basal prolongation of uropod longer than outer 10

9. False eave of telson with numerous (13) posterior projections . . . .*N. californiensis* (Manning, 1961).

9'. False eave of telson with rounded median projection, lateral margins not markedly subdivided . . . .*N. chilensis* (Dahl, 1954); . . . . Bahamonde, 1968.

10. False eave of telson with numerous (eight) posterior projections; anterolateral angles of rostral plate rounded . . . .*N. anomala* Manning, 1967.

10'. False eave of telson with rounded median projection, lateral margins not markedly subdivided; anterolateral angles of rostral plate sharp . . . .*N. decemspinosa* (Rathbun, 1910); Manning, 1961.

Lysiosquilla desaussurei (Stimpson, 1857)

Figure 2

Squilla desaussurei Stimpson, 1857, p. 503.

Lysiosquilla desaussurei. — Schmitt, 1940, p. 193. — Holthuis, 1967, p. 16 [other references].

Range. — Eastern Pacific region, where it was known only from the type-locality, off Mazatlán, México. The present records extend its distribution to Tangola-Tangola Bay, México, and Piedra Blanca Bay, Costa Rica.

Material examined. — Four specimens from two stations:

México

Tangola-Tangola Bay; 15°45′40″N, 96°06′05″W; Station 196, light; 8-12 December 1937; two males, one female.

Costa Rica

Piedra Blanca Bay; 09°51′47″N, 85°29′56″W; Station 208 L-1; 1 February 1938; one male.

Measurements. — Males, TL 68-86 mm; female, TL 84 mm. Other measurements of all four specimens are as follows:

Station	196	208	196	196
Sex	♂	♂	♂	♀
Total Length	68	84	86	63
Carapace Length	12.1	13.9	14.9	11.4
Cornea Width	4.3	5.0	5.1	4.5
Rostral Plate Length	2.9	3.6	3.8	2.9
Rostral Plate Width	3.8	4.7	4.8	3.5
Antennal Scale Length	6.4	7.5	8.2	5.8
Antennal Scale Width	2.0	2.0	2.5	1.5
Propodus Length	15.9	18.0	20.1	14.3
Fifth abdominal somite Width	15.1	16.8	18.7	13.7
Telson Length	10.5	12.5	14.0	9.8
Telson Width	14.0	16.1	16.9	12.7
Uropodal Endopod Length	6.0	6.8	7.5	5.2
Uropodal Endopod Width	2.6	2.8	3.3	2.3
Corneal Index	281	278	292	253
Propodal Index	761	772	741	797
Antennal Scale L/W ratio	320	375	328	387
Uropodal Endopod L/W ratio	231	243	227	226

Diagnosis. — Rostral plate cordiform, broader than long, with median carina. Antennal protopod with anterolaterally directed spine above articulation of antennal peduncle. Antennal scale slender, length more than three times greatest width, outlined in black. Dactylus of raptorial claw with twelve teeth. Ventral keel of eighth thoracic somite acute, sharp in some specimens, directed posteriorly. Posterior two abdominal somites, telson and proximal segment of uropod with dorsal tubercles and spinules. Uropod with slender ventral spine at articulation of endopod.

Color. — Overall color pattern, as in most species of *Lysiosquilla,* barred. Antennal scale dark, outlined in black. Three broad dark bands on carapace, posteriomost darkest. Merus of claw with narrow distal black bar. Body segments each with broad anterior dark band and narrower posterior black line. Dorsal surface of telson with median and submedian black spots. Basal segment of uropod dark proximally, light distally; uropodal exopod with black spot on articulation of distal segments, distal half of distal segment light; proximal third of endopod light, distal two-thirds black.

Remarks. — The rediscovery of Stimpson's species, which was known only from the type taken off Mazatlán, Mexico, is among the more important of the carcinological findings of the *Zaca* Eastern Pacific Expeditions. *L. desaussurei* is a distinctive species which resembles the western Atlantic *L. scabricauda* (Lamarck, 1818) and the eastern Atlantic *L. hoevenii* (Herklots, 1851) in having the dorsal surface of the posterior portion of the body roughened with spinules, tubercles, and granules. It differs from both of those species in having a sharper ventral keel on the eighth thoracic somite and in having a ventral spine on the basal segment of the uropod at the articulation of the endopod.

The dorsal roughness of the posterior portion of the body is not so well developed in these shall specimens as it is in adult specimens of *L. scabricauda* (see Manning, 1969) from the western Atlantic. There are a few blunt spinules on the posterolateral margins of the fifth abdominal somite, and the sixth abdominal somite is ornamented with an incomplete anterior line of tubercles, a few dorsolateral tubercles, and several posterior spinules. The lateral portions of the dorsal surface of the telson are pitted and roughened, but not strongly tuberculate. All specimens have a dorsal patch of tubercles and spinules on the basal segment of the uropod; in some specimens these spinules may be arranged in a curved row leading to the large distal fixed spine on that segment.

All of the specimens were taken at night light stations; specimens were collected by dip net at a submerged light.

To my knowledge, this species has never been illustrated. Its diagnostic features are shown here in Figure 2.

FAMILY SQUILLIDAE

Key to Genera and Species of Squillidae from the Eastern Pacific Region

1. Submedian teeth of telson with movable apices 2

1′. Submedian teeth of telson with fixed apices 7
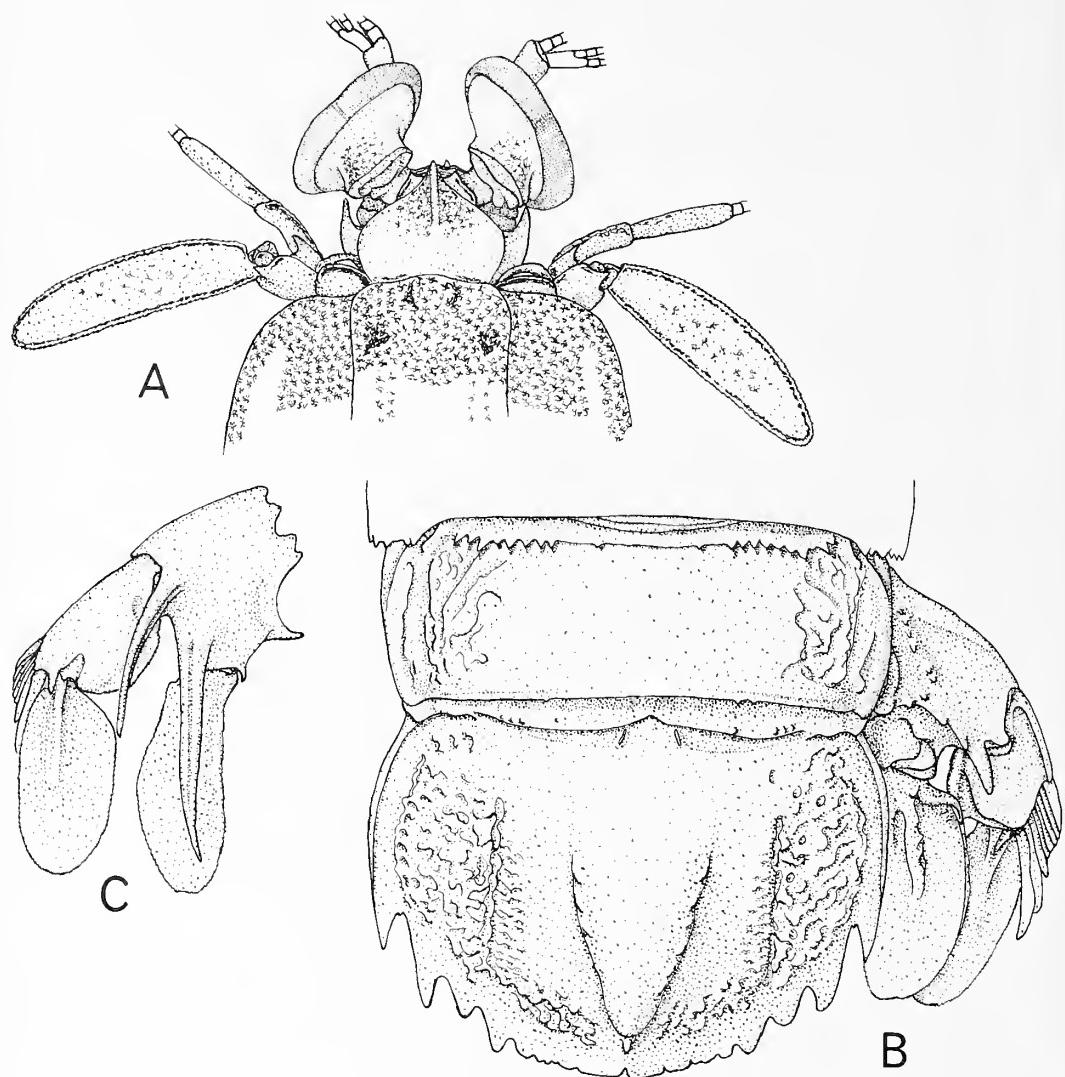
Figure 2. *Lysiosquilla desaussurei* (Stimpson), male, TL 84 mm, Piedra Blanca Bay: A, anterior portion of body; B, sixth abdominal somite, telson, and uropod; C, uropod, ventral view. (Setae omitted).

2. Dactylus of raptorial claw with six or more teeth; ocular scales produced into erect dorsal spines; *Pterygosquilla* Hilgendorf . 3

2'. Dactylus of raptorial claw with four to five teeth; ocular scales rounded; *Meiosquilla* Manning 4

3. Abdomen lacking submedian carinae; post-anal keel absent . . . . *P. gracilipes* (Miers, 1881); Schmitt, 1940; Bahamonde, 1968.

3'. Abdomen with submedian carinae; postanal keel present . . . . *P. armata armata* (H. Milne-Edwards, 1837); Schmitt, 1940; Bahamonde, 1968; Manning, 1969.

4. Anterolateral angles of carapace with spines . . . . *M. polita* (Bigelow, 1891).

4'. Anterolateral angles of carapace unarmed 5

5. Cornea anteriorly emarginate, appearing scalloped; antennules with geniculate spines . . . . *M. oculinova* (Glassell, 1942).

5'. Cornea bilobed, not anteriorly scalloped; antennules lacking geniculate spines 6

6. Dorsal surface of telson with carinae in addition to median carina and carinae of marginal teeth . *M. swetti* (Schmitt, 1940).

6'. Dorsal surface of telson ornamented only with median carina and carinae of marginal teeth . . *M. dawsoni* Manning, 1970.

7. No more than three epipods present; eye-stalks dilated, eyes flask-shaped; *Cloridopsis* Manning; *C. dubia* (H. Milne-Edwards, 1837); Schmitt, 1940; Manning, 1969.

7'. More than three epipods present; eyes T-shaped, stalk not dilated; *Squilla* Fabricius 8

8. Ischium of raptorial claw with ventrally directed spine; four epipods present . . . . *S. aculeata aculeata* Bigelow, 1893.

8'. Ischium of raptorial claw unarmed; five epipods present 9

9. Prelateral lobe of telson spined . . . . *S. bigelowi* Schmitt, 1940.

9'. Prelateral lobe of telson, if present, unarmed 10

10. Potanal keel of telson produced into posterior spine . . . . *S. biformis* Bigelow, 1891; Schmitt, 1940.

10'. Postanal keel of telson unarmed 11

11. Submedian carinae of sixth abdominal somite only with posterior spines 12

11'. Submedian carinae of fifth and sixth abdominal somites with posterior spines . . 14

12. Median carina of carapace, anterior to cervical groove, with well formed anterior bifurcation; rostral plate with median carina

. . . . *S. mantoidea* Bigelow, 1893; Bigelow, 1894.

12'. Median carina of carapace, anterior to cervical groove, lacking anterior bifurcation; rostral plate lacking median carina 13

13. Intermediate carinae of carapace extending to anterior margin; lateral processes of sixth and seventh thoracic somites acute posterolaterally; telson with dorsal tubercles lateral to median carina . . . . *S. hancocki* Schmitt, 1940.

13'. Intermediate carinae of carapace not extending to anterior margin; lateral processes of sixth and seventh thoracic somites produced into posterolateral spine; telson lacking dorsal tubercles lateral to median carina . . . . *S. tiburonensis* Schmitt, 1940.

14. Median carina of carapace, anterior to cervical groove, with anterior bifurcation; submedian carinae of fourth, fifth, and sixth abdominal somites with posterior spine . . . . . *S. panamensis* Bigelow, 1891.

14'. Median carina of carapace, anterior to cervical groove, lacking anterior bifurcation; submedian carinae of fifth and sixth abdominal somites with posterior spines . . . . *S. parva* Bigelow, 1891.

Meiosquilla polita (Bigelow, 1891)

Squilla polita Bigelow, 1891, p. 93. — Schmitt, 1940, p. 146, fig. 2. — Manning, 1968, p. 125 [listed; transferred to *Meiosquilla*].

Range. — Eastern Pacific region, from Monterey Bay, California, to off Abreojos Point, Lower California, México.

Material examined. — México; Lower California, east of Cedros Island; 28°21'N, 115°-11'W; Station 126 D-11; 80 meters; 22 May 1936; four foot dredge; one male.

Measurements. — Broken male, CL 10.3 mm.

Meiosquilla oculinova (Glassell, 1942)

Squilla oculinova Glassell, 1942, p. 53, fig. 7. — Manning, 1968, p. 125 [listed; transferred to *Meiosquilla*].

Range. — Eastern Pacific region, where it was known only from the type-locality, off Manzanillo, México.

Material examined. — México; Chamela Bay; 17-20 November 1937; food of *Evoplites;* one male.

Measurements. — Broken male, CL 6.9 mm.

Remarks. — This striking species, characterized by the anteriorly emarginate eyes and the peculiar curved spines on the antennular peduncle, has not been recorded since its original description.

Meiosquilla swetti (Schmitt, 1940)

Squilla swetti Schmitt, 1940, p. 146, fig. 3. —
    Manning, 1968, p. 125 [listed; transferred to
    Meiosquilla].

Range. — Eastern Pacific region, where it was
known only from the type-locality, off Petatlán
Bay, México. The specimens reported here ex-
tend the range southward to Tangola-Tangola,
México, and Corinto, Nicaragua.

Material examined. —Two specimens from
two stations:

México

Tangola-Tangola Bay; 15°45'N-15°45'22"N,
96°05'34"W-96°05'51"W; Station 196 D-16, 17;
mud; 29-42 meters; 3 December 1937; four foot
dredge; one male.

Nicaragua

Corinto; 12°27'19"N, 87°11'39"W; Station
200 D-20; mangrove leaves; 2.7 meters; 7 Janu-
ary 1938; two foot dredge; one female.

Measurements. — Male, TL 19 mm; female,
TL 31 mm.

Color. — The color pattern of this pretty spe-
cies was not mentioned in the original account;
the pattern is well preserved in the specimens
taken by the *Zaca*. Body marked with numerous
light brown chromatophores. Antennular ped-
uncle with irregular brown markings. Antennal
scale distally outlined in brown. Merus of claw
with distal dark bar and proximal dark spot on
dorsal depression. Carapace outlined with light
brown chromatophores, with traces of two an-
terior diffuse dark bars and a darker posterior
bar. Posterior three thoracic and anterior five
abdominal somites with dark anterolateral line,
rectangular median dark patch, posterior black
line, and black posterolateral spot. Carinae of
sixth abdominal somite and marginal teeth of
telson dark. Telson with broad, irregular bar
extending from lateral tooth to apex of median
carina. Uropod outlined with dark pigment, dis-
tal segment of exopod and endopod black with
lighter central area.

Remarks. — These specimens agree well with
Schmitt's original account in most respects.
There is some variability in the configuration of
the dorsal carinae of the telson. The long sub-
median carinae may be subdivided into three
portions, the anteriormost largest, and there may
be only four carinae, the third longest, lateral
to the submedians; in the type there were four
or five carinae lateral to the submedians. The
longest lateral carina may also be subdivided
into two or more portions.

Meiosquilla dawsoni Manning, 1970

Meiosquilla dawsoni Manning, 1970, p. 102,
    fig. 3.

Range. — Eastern Pacific region, where it was
known from Panama and off Guaymas, México;
it has not been recorded previously from Costa
Rica.

Material examined. — Costa Rica; Port Cu-
lebra; 10°36'22"N, 85°41'08"W; Station 206
D-3; sandy mud; 25.5 meters; 30 January 1938;
four foot dredge; one male.

Measurements. — Male, TL 19 mm.

Remarks. — This small specimen is consider-
ably smaller than the types, both of which were
larger than 30 mm; the carinae of the sixth
abdominal somite and the margins of the telson
show no signs of the inflation present in the
types. In other respects, this specimen agrees
well with the original account of the species.

Squilla aculeata aculeata Bigelow, 1893

Squilla aculeata Bigelow, 1893, p. 101. —
    Schmitt, 1940, p. 158, fig. 9 [older references].
    — Manning, 1968, p. 129 [listed]. — Baha-
    monde, 1968, p. 116.

Range. — Eastern Pacific region, from Tea-
capan, Sinaloa, México, several localities off
Panama, and Iquique, Chile. It has not been
recorded previously from Nicaragua or El
Salvador.

Material examined. — Three specimens from
two stations:

Nicaragua

Monypenny Point, Gulf of Fonseca; 13°03'-
30"N, 87°30'20"W; Station 199 D-4; mud; 12.8
meters; 24 December 1937; four foot dredge;
one female.

El Salvador

La Union, Gulf of Fonseca; 13°19'08"N,
87°47'30"W; Station 199 D-12; mud; 9.1 me-
ters; 27 December 1937; four foot dredge; one
male, one female.

Measurements. — Male, TL 65 mm; females,
TL 35 and 82 mm. Corneal Indices are sum-
marized in Table 2.

Remarks. — In a report on some stomatopods
from the Gulf of Guinea, Manning (in press)
showed that the eastern Atlantic *Squilla calmani*
Holthuis, 1959, must be considered a subspecies
of *Squilla aculeata* Bigelow from the eastern
Pacific.

Squilla hancocki Schmitt, 1940

Squilla hancocki Schmitt, 1940, p. 160, fig. 10.
    — Manning, 1968, p. 129 [listed].

Range. — Eastern Pacific region, from off
Petatlán and Tangola-Tangola Bays, México,
and off Cape San Francisco, Ecuador.

Material examined. — Fourteen specimens
from three stations:

TABLE 2. CORNEAL INDICES OF SPECIMENS OF *Squilla* FROM THE *Zaca* COLLECTIONS.

Carapace Length	S. aculeata aculeata	S. hancocki	S. panamensis	S. parva	S. tiburonensis
5.0				353-357 (2)	
6.0		333		321-394 (6)	
7.0	384				
8.0					
9.0					
10.0					
11.0			303		
12.0			300		328
13.0		350-353 (2)	302		
14.0	453	349	326		363
15.0			317-319 (2)		
16.0					
17.0	483		329		
18.0			342		
19.0			333-338 (2)		
20.0			328-343 (2)		

México

Tenacatita Bay; 19° 14′ 30″N - 19° 15′ 30″N, 104°51′W-104°51′30″W; Station 183 D-2, 3; muddy sand, sandy mud; 54-73 meters; 21 November 1937; four foot dredge; seven females.

Tangola-Tangola Bay; 15°45′N-15°45′22″N, 96°05′34″W-96°05′51″W; Station 196 D-16, 17; mud; 29-42 meters; 13 December 1937; four foot dredge; two males.

Tangola-Tangola Bay; 15°44′58″N, 96°05′-13″W; Station 196 D-18; mud; 55 meters; 13 December 1937; four foot dredge; three males, two females.

Measurements. — Males, TL 20-68 mm; females, TL 32-64 mm. Corneal Indices are summarized in Table 2.

Remarks. — The characteristic color pattern described by Schmitt (1940) is visible in most of the specimens. The smaller ones lack the dorsal tubercles of the telson, but tubercles or lines of tubercles are present on the telson in all that are longer than about 50 mm in total length. All of the specimens, including the smallest, have the characteristic angled lateral process of the seventh thoracic somite.

The four smallest specimens, 20, 24, 30, and 32 mm in length are probably postlarvae. The body carination and spination is reduced, the anterolateral angles of the carapace are unarmed, and the submedian teeth of the telson are provided with movable apices. The size range of specimens exhibiting postlarval characters suggests that in this species these characters may be retained for more than one molt after the pelagic larval life is completed.

In the two large males, TL 63-68 mm, the carinae on the posterior part of the body as well

as the dorsal carinae are inflated, indicating that these specimens are mature.

Squilla tiburonensis Schmitt, 1940

Squilla tiburonensis Schmitt, 1940, p. 165, fig. 11. — Manning, 1968, p. 129 [listed].

Range. — Eastern Pacific region, where it is known only from localities in the Gulf of California.

Material examined. — Three specimens from two stations:

México

Gulf of California, Santa Inez Bay; 26°59′-30″N, 111°59′W; Station 141 D-4; 36 meters; 10 April 1936; four foot dredge; one male.

Gulf of California, Santa Inez Bay; 26°54′N, 111°53′W; Station 143 D-5; 33 meters; 13 April 1936; four foot dredge; one male, one female.

Measurements. — All specimens damaged. Male, CL 11.8 mm; female, CL 13.8 mm. Corneal Indices are summarized in Table 2.

Squilla panamensis Bigelow, 1891

Squilla panamensis Bigelow, 1891, p. 94. — Schmitt, 1940, p. 166, fig. 13. — Manning, 1968, p. 129 [listed].

Range. — Eastern Pacific region, from scattered localities between Petatlán Bay, México and Cape Corrientes, Colombia.

Material examined. — Thirteen specimens from five stations:

México

Thirteen miles west of Mazatlán; 23°12′N, 106°40′W; Station 155 D-1; 102 meters; 28 April 1936; four foot dredge; one male, two females.

Tenacatita Bay; 19° 14′ 30″N - 19° 15′ 30″N,

104°51′W-104°51′30″W; Station 183 D-2, 3; sandy mud, muddy sand; 54-73 meters; 21 November 1937; four foot dredge; one male.

Costa Rica

Off Ballenas Bay, Gulf of Nicoya; 09°42′-10″N-09°44′52″N, 84°51′08″W-84°51′25″W; Station 213 D-11, 13-15; mud; 63.7-73 meters; 25 February 1938; four foot dredge; three males, two females.

Off Ballenas Bay, Gulf of Nicoya; 09°42′N, 84°56′W; Station 213 D-17; mud; 63.7 meters; 25 February 1938; four foot dredge; one male, one female.

Panama

Gulf of Chiriqui; 07°54′45″N, 82°04′32″W; Station 221 D-1; sandy mud; 64 meters; 13 March 1938; four foot dredge; two males.

Measurements. — Males, TL 58-101 mm; females, TL 52-95 mm. Corneal Indices are summarized in Table 2.

Remarks. — The submedian carinae of the fourth abdominal somite are spined posteriorly in all specimens. The bases of the marginal teeth of the telson show no signs of inflation in the smaller males, but specimens 65 to 90 mm long exhibit slight inflation, and larger specimens, 95 mm long or more, have the bases of the marginal teeth noticeably inflated. Some of the larger specimens have traces of one or two small tubercles on the dorsal surface of the telson; these tubercles, when present in *S. panamensis,* are never so well developed as in specimens of *S. hancocki.*

Squilla parva Bigelow, 1891

Squilla parva Bigelow, 1891, p. 94. — Schmitt, 1940, p. 168, fig. 14. — Manning, 1968, p. 129 [listed].

Range. — Eastern Pacific region, from scattered localities between Manzanillo, México, and Cape San Francisco, Ecuador. It has not been recorded previously from Costa Rica or El Salvador.

Material examined. — Twelve specimens from five stations:

México

Tangola-Tangola Bay; 15°45′N-15°45′22″N, 96°05′34″W-96°05′51″W; Station 196 D-16, 17; mud; 29-42 meters; 13 December 1937; four foot dredge; three females.

El Salvador

La Libertad; 13°25′50″N-13°27′20″N, 89°-19′20″W; Station 198 D-1, 2; mud; 24-25 meters; 16 December 1937; four foot dredge; three males.

Meanguera Island, Gulf of Fonseca; 13°08′N,

87°43′W; Station 199 D-1; mud, crushed shell; 29 meters; 23 December 1937; four foot dredge; one male.

Costa Rica

Port Culebra; 10°36′22″N, 85°41′08″W; Station 206 D-3; sandy mud; 25.5 meters; 30 January 1938; four foot dredge; one female.

Panama

Bahia Honda; 07°45′35″N-07°45′51″N, 81°32′18″W-81°32′21″W; Station 222 D-1, 5; rocks, dead coral, mud, shells, leaves; 5.4-20 meters; 18 March 1938; two foot dredge; three males, one female.

Measurements. — Males, TL 22-32 mm; females, TL 19-33 mm. Corneal Indices are summarized in Table 2.

Family Gonodactylidae

Key to Genera and Species of Gonodactylidae from the Eastern Pacific Region

1. Ischiomeral articulation of raptorial claw terminal; merus of claw grooved inferiorly throughout its length 2

1′. Ischiomeral articulation of raptorial claw not terminal, merus projecting posteriorly beyond articulation; inferior groove on merus of claw incomplete; *Gonodactylus* Berthold 9

2. Dactylus of raptorial claw unarmed; sixth abdominal somite unarmed posteriorly; *Hemisquilla* Hansen 3

2′. Dactylus of raptorial claw with teeth; sixth abdominal somite with armed carinae or with posterior spines 4

3. Mandibular palp usually three segmented (25% two segmented; 75% three segmented); length/width ratio of rostral plate usually high (mean 1.34); northern population, southern California to Panama... **H. ensigera californiensis* Stephenson, 1967.

3′. Mandibular palp two or three segmented (45% two segmented, 55% three segmented); length/width ratio of rostral plate usually low (mean 1.17); southern population, Chile . . . .*H. ensigera ensigera* (Owen, 1832); Stephenson, 1967; Bahamonde, 1968.

4. Inner spine of basal prolongation of uropod longer than outer; dactylus of raptorial claw with more than four teeth; *Eurysquilla* Manning 5

4′. Inner spine of basal prolongation of uropod shorter than or subequal to outer; dactylus of raptorial claw with three teeth 6

5. Basal prolongation of uropod consisting of two spines, with at most rounded lobe on inner margin . . . . *E. veleronis* (Schmitt, 1940).

5'. Basal prolongation of uropod consisting of two spines with series of spinules on inner margin . . . . *E. solari* Manning, 1970.

6. Basal prolongation of uropod with two spines, inner margin unarmed; *Pseudosquilla* Dana; *P. adiastalta* Manning, 1964.

6'. Basal prolongation of uropod with three spines, proximal smallest 7

7. Anterior five abdominal somites with prominent longitudinal carinae; telson with submedian denticles in adults; *Parasquilla* Manning; *P. similis* Manning, 1970.

7'. Anterior five abdominal somites lacking longitudinal carinae; telson lacking submedian denticles in adults; *Pseudosquillopsis* Serène 8

8. Lateral processes of sixth and seventh thoracic somites with posterolateral spine . . . . *P. marmorata* (Lockington, 1877); Manning, 1969a.

8'. Lateral processes of sixth and seventh thoracic somites rounded posterolaterally . . . . *P. lessonii* (Guérin, 1830); Schmitt, 1940; Bahamonde, 1968.

9. Carinae of telson unarmed dorsally, lacking dorsal tubercles or spinules (median and anterior submedian carinae often with posterior tubercle or spinule) 10

9'. Carinae of telson with dorsal spinules or tubercles 12

10. Telson of Bredini-type, with intermediate marginal teeth not widely set off from submedians, intermediate denticles set at or posteriorly to level of intermediate tooth . . . . *G. zacae*, new species.

10'. Telson of Oerstedii-type, with intermediate marginal teeth distinct and intermediate denticles recessed anteriorly 11

11. Median carina of telson with posterior spine; males 12 to 20 mm in length with median carina inflated, obscuring anchor . . . .*G. pumilus* Manning, 1970.

11'. Median carina lacking posterior spine; males 12 to 20 mm in length with normal median carina of telson, not noticeably inflated . . . .*G. oerstedii* Hansen, 1895; Schmitt, 1940; Manning, 1969.

12. Knob posterior to apex of median carina unarmed . . . . . *G. stanschi* Schmitt, 1940.

12'. Knob posterior to apex of median carina with two or more posterior tubercles or spinules 13

13. Accessory median carinae of telson short, not extending anteriorly for one-fourth length of median carina, fusing posteriorly with median carina to form anchor . . . .*G. festae* Nobili, 1901.

13'. Accessory median carinae of telson long, extending anteriorly almost to base of median carina 14

14. Knob with no more than two spinules; anterolateral angles of rostral plate spiniform . . . . . .*G. bahiahondensis* Schmitt, 1940.

14'. Knob with four or more spinules; anterolateral angles of rostral plate acute but not spiniform . . . . *G. lalibertadensis* Schmitt, 1940.

Hemisquilla ensigera californiensis
Stephenson, 1967

Hemisquilla stylifera. — Schmitt, 1940, p. 182, fig. 18a [other references].
Hemisquilla ensigera. — Manning, 1963a, p. 315.
Hemisquilla ensigera californiensis Stephenson, 1967, p. 15.

Range. — Eastern Pacific region, from southern California, México (including Gulf of California), and Panama. The nominal subspecies is known from off Chile, and a third subspecies, *H. e. australiensis* Stephenson, occurs off Australia (Stephenson, 1967).

Material examined. — Two specimens from two stations:

México

Arena Bank, Gulf of California; 23°20'N, 109°25'W; Station 136 D-18; 73 meters; 20 April 1936; four foot dredge; one male.

Panama

Gulf of Chiriqui; 07°52'45"N, 82°02'W; Station 221 D-4; sandy mud; 69 meters; 13 March 1938; four foot dredge; one male.

Measurements. — Males only examined, TL 90 and 104 mm.

Remarks. — Stephenson (1967) recognized the three Pacific populations (Californian, Chilean, and Australasian) as distinct subspecies, based on analyses of characters from samples from these three areas as well as on their apparent distinct, isolated geographic ranges. Characters used by Stephenson include the number of segments on the mandibular palp, the number of intermediate denticles (lobes) on the telson, and proportions of the rostral plate and the eye (see Table 3). As might be expected, all of the characters used by Stephenson overlap broadly; in spite of this, as he pointed out, the Chilean population appears to be as distinct from the Californian as it is from

the Australasian. Although the recognition of three subspecies is probably sound on a biological basis, the broad overlap of characters makes it difficult at best to identify a single specimen to the subspecific level on any basis other than geography. For the present, at least, it seems best to consider the single specimen from Panama recorded here, as well as the single, small (CL 6.0 mm) damaged specimen from Jicarita Island, Panama, reported by Stephenson (1967), but not identified to subspecies, with the Californian subspecies. The relatively large numbers of specimens from localities off Mexico reported by Stephenson suggest that the northern population of *H. ensigera* extends from southern California to at least northern Panama; the southern population is not known to occur north of Chile.

A note accompanying the specimen from station 136 identified it as a "giant purple-uropoded Squilla." Apparently members of both American subspecies have brightly colored uropods.

Eurysquilla veleronis (Schmitt, 1940)

Pseudosquilla veleronis Schmitt, 1940, p. 176, fig. 17. — Manning, 1963, p. 314 [listed; transferred to *Eurysquilla*].

Range. — Eastern Pacific region, where it has been recorded from Angeles Bay, Gulf of California, and Chacahua Bay and off Petatlán Bay, both Oaxaca, México.

Material examined. — México; Tangola-Tangola Bay; 15°44′58″N, 96°05′13″W; Station 196 D-18; mud; 55 meters; 13 December 1937; four foot dredge; one male, two females.

Measurements. — Male, TL 17 mm; females, TL 18 mm.

Remarks. — All three specimens are juveniles. Most adult features are discernible, but all three have submedian denticles on the telson; these denticles, apparently characteristic of postlarvae and juveniles of *Eurysquilla,* are not present in adults.

Pseudosquilla adiastalta Manning, 1964

Pseudosquilla oculata. — Schmitt, 1940, p. 173, fig. 15 [not *P. oculata* (Brullé)].

Pseudosquilla adiastalta Manning, 1964, p. 304, fig. 1.

Range. — Eastern Pacific region, from the Tres Marias Islands, México, to the Galapagos Islands, including Clarion and Clipperton Islands and localities off Panama and Colombia.

Material examined. — Eight specimens from four stations:

México

Sihuatenejo Bay; in coral; 24 November 1937; one male.

Port Guatulco; 15°44′54″N, 96°07′57″W; Station 195 D-15; in coral; 2.7 meters; 6 December 1937; diving; four females.

Costa Rica

Port Parker; in coral; 16-17 January 1937; one male.

Jasper Island; in coral; 22-25 February 1937; two males.

Measurements. — Males, TL 25-57 mm; females, TL 33-55 mm.

Pseudosquillopsis marmorata
(Lockington, 1877)

Squilla marmorata Lockington, 1877, p. 33.

Pseudosquilla lessonii. — Schmitt, 1940, p. 175, fig. 16 [part; not *P. lessonii* (Guérin, 1830)].

TABLE 3. MORPHOMETRIC DATA FOR *Hemisquilla ensigera.*

	H. ensigera ensigera Chile (Stephenson, 1967)	*H. ensigera californiensis* California (Stephenson, 1967)	136 D-18	221 D-4
Length/Width Rostral Plate:				
Mean	1.17	1.34	1.38	1.21
Range	1.10-1.29	1.10-1.54	–	–
Length Carapace/Length Rostral Plate:				
Mean	4.54	4.05	3.88	4.28
Range	4.10-5.15	3.50-4.57	–	–
Cornea Width/Cornea Length:				
Mean	1.27	1.32	1.29	1.19
Range	1.18-1.38	0.95-1.55	–	–
Eye Length/Cornea Length:				
Mean	1.51	1.46	1.47	1.38
Range	1.41-1.65	1.12-1.92	–	–

Pseudosquillopsis marmorata. — Manning, 1969a, pp. 527, 531, figs. 1, 3 [postlarvae and juveniles].

Range. — Eastern Pacific region, from southern California, the Gulf of California, and the Galapagos Islands.

Material examined. — One specimen from one station:

México

Arena Bank, Gulf of California; 23°27′N, 10°24′W; Station 136 D-30; 1 May 1936; 64 meters; four foot dredge; one postlarval female.

Measurements. — Female postlarva TL 28 mm.

Remarks. — Manning (1969a) pointed out the differences between the closely related *P. marmorata* and *P. lessonii* and showed that the species could be distinguished even at the postlarval stage. The specimen reported here agrees well with the account given by me of the postlarvae, except that the posterior spine on the lateral process of the seventh thoracic somite is even more prominent than shown in figure 1 in the 1969 paper.

GENUS *Gonodactylus* BERTHOLD

The American species of *Gonodactylus* are particularly troublesome for several reasons. They are very similar, all apparently having been derived from a single stock; all share the accessory intermediate carina on the telson and by this feature can be distinguished from all Indo-west Pacific species of the genus, in excess of 20 in number. Our knowledge of ontogenetic changes in morphology of *Gonodactylus* is limited. Some American species have dorsal tubercles or spinules on the telson and are easily recognizable. Many of the eastern Pacific species of the genus have acute or even spiniform anterolateral angles on the rostral plate; these angles are rounded in all western Atlantic species. Although it is known that the numbers of characters available for use in species distinction is very limited, but some of those which are generally reliable become modified in the adult males; for example, some features of telson morphology are distorted by secondary sexual changes in adult males.

Analysis of a large series of *Gonodactylus* for a review of the western Atlantic species (Manning, 1969) showed that one feature of telson morphology, the shape and position of the intermediate teeth and denticles, could be valuable in species recognition. Schmitt (1940) was the first to point out the utility of these features. He referred to two basic telson types which he found in *G. oerstedii* as the Atlantic and the Pacific types of telson; he noted that these particular telson shapes were not necessarily restricted to Atlantic or Pacific specimens, but that both types of telson could be found in specimens from either ocean.

Manning (1969) considered that these two types of telson reflected specific differences and that the Atlantic species could be characterized by two telson types. Some species have a telson, designated by him as the Oerstedii-type, in which the intermediate marginal teeth are subparallel to and distinct from the submedian teeth and in which the intermediate marginal denticles are recessed anteriorly, that is, set anterior to the apex of the intermediate tooth. Other species share a telson referred to as the Bredini-type, in which the longitudinal axes of the intermediate teeth are convergent posteriorly with the longitudinal axes of the submedian teeth and in which the intermediate denticles are set at or posterior to the level of the apex of the intermediate tooth.

Both of these telson types occur in the eastern Pacific species of *Gonodactylus,* and a new species with a Bredini-type telson is described below. The remainder of the eastern Pacific species recorded here all have the Oerstedii-type telson; in addition, they all have acute anterolateral angles on the rostral plate and dorsal tubercles or spinules on the telson.

All of the characters discussed by Schmitt (1940) in relation to eastern Pacific *Gonodactylus* appear to apply to the specimens taken by the *Zaca* Expeditions, including the shape of the ocular scales, the rostral plate, and telson.

The Abdominal Width-Carapace Length Index, introduced by Manning (1969), has been summarized in tabular form (Table 4) for all of the specimens reported here. The indices overlap broadly, as they did for most western Atlantic species, but it appears that adults of *G. stanschi* have proportionally narrower abdomens than do those of *G. festae.*

None of the eastern Pacific specimens taken by the *Zaca* cruises are particularly large, but all of the specimens of *G. zacae* are unusually small, suggesting that it, like the western Atlantic *G. torus* Manning, is a dwarf species.

Gonodactylus zacae, new species

Figure 3

Holotype. — Male, TL 30 mm; Port Guatulco, México; 15°44′30″N-15°44′35″N, 96°07′-56″W-96°08′W; Station 195 D-7, 8; rocks, sand, algae; 8.2-11 meters; 5 December 1937; two foot dredge; AMNH 14044.

Paratypes. — Fifty-six specimens from seven stations:

TABLE 4. SUMMARY OF ABDOMINAL WIDTH-CARAPACE LENGTH INDICES
(AWCLI) FOR SPECIMENS OF *Gonodactylus*.

Carapace Length		*bahiahondensis*	*festae*	*lalibertadensis*	*stanschi*	*zacae*
3	mean	—	—	—	824	873 (6)
	range				—	862-885
4	mean	790 (3)	—	—	—	796 (9)
	range	775-805				767-857
5	mean	802 (3)	—	—	—	790 (12)
	range	788-822				740-846
6	mean	777 (2)	—	833	790 (2)	777 (8)
	range	766-787		—	780-800	745-818
7	mean	753 (3)	—	—	769 (5)	—
	range	740-770			746-792	
8	mean	759 (4)	773 (2)	768	734	—
	range	740-769	768-778	—	—	
9	mean	—	780 (2)	—	733 (3)	—
	range		777-783		713-766	

México

Port Guatulco; 15°44′45″N, 96°07′53″W; Station 195 D-3; sand, crushed shell; 6.3 meters; 4 December 1937; two foot dredge; one male, two females (USNM).

Port Guatulco; 15°44′40″N, 96°07′53″W; Station 195 D-4; sand, algae; 8.2 meters; 4 December 1937; two foot dredge; five males, five females, AMNH 14045, four males (USNM).

Port Guatulco; 15°44′50″N, 96°08′09″W; Station 195 D-5; sand, algae; 3.6 meters; 5 December 1937; two foot dredge; eleven males, six females, AMNH 14045.

Port Guatulco; 15°44′45″N, 96°08′05″W; Station 195 D-6; sand, algae; 5.4 meters; 5 December 1937; two foot dredge; two males, one female, AMNH 14045.

Port Guatulco; data as in holotype; Station 195 D-7,8; five males, eight females, AMNH 14045.

Port Guatulco; 15°44′27″N, 96°07′57″W; Station 195 D-14; coral; 7.3 meters; 6 December 1937; two foot dredge; two males, three females (in two lots), AMNH 14045.

Other material. — Twenty-eight specimens from seven stations:

México

Santa Inez Bay, Gulf of California, Baja California; 27°00′30″N, 111°58′30″W; Station 141 D-3; 33 meters; 10 April 1936; four foot dredge; one male.

Arena Bank, Gulf of California; 23°27′N, 109°24′W; Station 136 D-30; 64 meters; 1 May 1936; four foot dredge; one male.

Costa Rica

Port Parker; 12-23 January 1938; one female.

Port Parker; 10°55′06″N, 85°48′53″W; Station 203 D-4; gravel, algae; 12.8 meters; 12 January 1938; two foot dredge; one male.

Port Parker; 10°55′43″N, 85°49′37″W; Station 203 D-7; shells, algae; 16.4-19.1 meters; 22 January 1938; two foot dredge; four males, four females.

Port Parker; 10°55′51″N, 85°49′52″W; Station 203 D-9; coral; 2.7-7.2 meters; 22 January 1938; two foot dredge; six males, seven females.

Panama

Bahia Honda; 07°45′35″N-07°45′51″N, 81°32′18″W-81°32′21″W; Station 222 D-1,5; rocks, dead coral, mud, shells, leaves; 5.4-20 meters; 18 March 1938; two foot dredge; three males.

Measurements. — Males, TL 9-36 mm; females, TL 8-32 mm. Other measurements of male holotype, TL 30 mm: carapace length, 5.9 mm; fifth abdominal somite width, 4.8 mm; telson length, 4.4 mm, width, 4.5 mm.

Diagnosis. — Anterolateral angles of rostral plate acute but broadly rounded. Ocular scales erect, small, rounded or squarish, not projecting laterally. Lateral process of sixth thoracic somite broadly rounded anteriorly, more truncate posteriorly. Lateral process of seventh thoracic somite truncate anteriorly and posteriorly, slightly more rounded anteriorly, narrower than process of sixth somite. Lower portion of posterior margin of pleura of anterior four abdominal somites straight or nearly so. Anterior five abdominal somites unarmed posterolaterally. Sixth abdominal somite with six

longitudinal carinae, very swollen in large speci-mens; each carina with posterior spine in small specimens, apices with at most blunt tubercle in adults. Abdominal Width-Carapace Length Indices summarized in Table 4. Telson longer than broad or with length and width subequal, of Bredini type, appearing triangular, without dorsal tubercles or spinules; median carina in-flated in most specimens, flask-shaped in juveniles, occasionally with blunt posterior tubercle; accessory median carinae short, ex-tending anteriorly to about midlength of median carina (extending farther anteriorly in small males than in females), fusing posteriorly with median carina to form anchor; anchor com-pletely obliterated by inflation of median carina in adult males; knob rounded, not prominent, unarmed, often fused with inflated median carina; anterior submedian carinae inflated, each with at most a posterior dimple; carinae of sub-median teeth inflated, rounded dorsally; inter-mediate, accessory intermediate and marginal carinae well defined, not markedly inflated; submedian teeth with poorly formed shelf on inner margin, lacking anterior angled promi-nence; low, oblique prominences divergent posteriorly from under knob; submedian teeth convergent posteriorly, movable apices usually absent, numerous small submedian denticles present; intermediate teeth blunt, apices short and rounded, longitudinal axes of teeth con-vergent with those of submedian teeth; lateral tooth distinct but poorly formed, apex blunt; one rounded intermediate denticle present, set posterior to apex of intermediate tooth; lateral denticle absent. Uropodal endopod broad, inner margin straight or nearly so.

Color. — The color pattern is not well pre-served in any of the specimens. In general, males differ from females in having more dark pigment on the ventral surface of the body: the proximal segments of the posterior three maxillipeds and the male copulatory tubes are black, and the thoracic sterna of the posterior three thoracic somites and the bases of the walking legs are dark grey. Color notes accompanying two of the lots indicate that the species is scarlet (male, Station 141 D-3) or vermilion (male, Station 136 D-30) in life.

Remarks. — *Gonodactylus zacae,* new spe-cies, is the eastern Pacific counterpart of *G. bredini* Manning from the western Atlantic region; the new species can be distinguished from *G. bredini* by the longer accessory median carinae, which in the new species extend an-teriorly to near the midlength of the median carina. It may also be a broader species; the Abdominal Width-Carapace Length Indices of the smaller specimens (862-885) do not overlap those of *G. bredini* of similar size (756-851). The intermediate marginal denticles on the tel-son in *G. zacae* are always set posterior to the apex of the intermediate tooth, whereas they may be situated more anteriorly in *G. bredini.* Finally, the new species is a smaller species, not known to exceed 36 mm in length; *G. bredini* may attain a length of 75 mm.
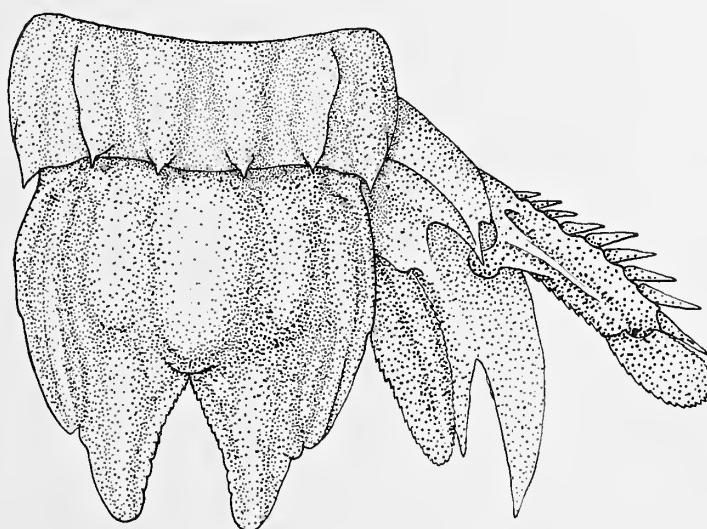

FIGURE 3. **Gonodactylus zacae, new species,** male holotype, TL 30 mm, Port Guatulco, Mexico: sixth abdominal somite, telson, and uropod (setae omitted).

The movable apices of the submedian teeth of the telson may be present in smaller specimens of *G. zacae;* they are not present in specimens larger than 20 mm.

Large males show marked secondary sexual characters, particularly in the inflation of the carinae of the sixth abdominal somite and the median carina of the telson. The inflation of these carinae is visible in specimens as small as 20 mm, and it is usually well developed at a size of 25 mm. In larger individuals, the carinae of the sixth abdominal somite are very inflated and are rarely provided with posterior spines. The median carina of the telson can completely obliterate the accessory medians and the anchor as well; it resembles the median carina found in adult specimens of *G. torus* Manning.

The specimens identified by Schmitt (1940) as *G. oerstedii* with a Pacific type telson may prove to be a member of the species described here; his material will be restudied in a planned review of the eastern Pacific stomatopods.

Gonodactylus stanschi Schmitt, 1940

Gonodactylus stanschi Schmitt, 1940, p. 215, fig. 30.

Range. — Eastern Pacific region, where it had been recorded from several localities between Angel de la Guardia Island, Gulf of California, to Tangola-Tangola, México; the localities recorded here are within the known range of the species.

Material examined. — Thirteen specimens from two stations:

México

Sihuatenejo Bay; in coral; 24 November 1937; two males, five females.

Port Guatulco; 15°44'54"N, 96°07'57"W; Station 195 D-15; coral; 2.7 meters; two males, four females.

Measurements. — Males, TL 19-36 mm; females, TL 12-41 mm. Abdominal Width-Carapace Length Indices are summarized in Table 4.

Color. — Faded in most specimens, but several appear banded with black chromatophores, and some show traces of dark median and lateral patches on the sixth thoracic somite as well as a median dark patch on the first abdominal somite.

Remarks. — This species can be distinguished readily from the other members of *Gonodactylus* bearing spinules on the telson by the reduced number of spinules. The accessory median carinae are always armed posteriorly with a single spine; the anchor found in *G. festae* is not developed. The knob is unarmed, and the dorsal carinae of the submedian teeth are ornamented with no more than one spinule or tubercle; in large specimens the tubercles may be replaced by a dimple in the surface of the carina. The accessory intermediate carinae are unarmed. In large males, 35 mm or more in length, the swollen median carina may completely obliterate the accessory submedian; the posterior denticles of the latter may be represented by obscure tubercles. The dorsal patterns of spinulation of *G. stanschi* are summarized in Table 5.

Gonodactylus festae Nobili, 1901

Gonodactylus festae Nobili, 1901, p. 53. — Schmitt, 1940, p. 220, fig. 32 [other references].

Range. — Eastern Pacific region, from the Gulf of Fonseca, El Salvador to Santa Elena Bay, Ecuador; it had not been recorded north of Salinas Bay, Costa Rica.

Material examined. — Four specimens from four stations:

El Salvador

Fumerole, Gulf of Fonseca; December 1937; one male.

Costa Rica

Port Parker; 12-23 January 1938; one male.

Piedra Blanca Bay; tidepool; 1-6 February 1938; one male.

TABLE 5. PATTERN OF DORSAL SPINATION OF TELSON IN *Gonodactylus* FROM THE EASTERN PACIFIC REGION.

	bahiahondensis	*festae*	*lalibertadensis*	*stanschi*
Median Carina	1	0 − 1	1	1
Accessory Median	0 − 1	0 − 4	1	1
Anchor	absent	3 − 4	absent	absent
Knob	2	4 − 5	4	−
Anterior Submedian	1 + 1 − 2	1 + 0 − 3	1 + 1	1
Submedian	1 − 3	4 − 6	2	0 − 1
	in 1 row	in 2 rows	in 1 row	
Accessory Intermediate	0 − 3	3	2	−
Lateral Denticle	−	+	−	−

Panama

Bahia Honda; under stone, low tide; 13-19 March 1938; one male.

Measurements. — Males only examined, TL 38-44 mm. Abdominal Width-Carapace Length Indices are summarized in Table 4.

Color. — Faded in most specimens, but traces of the pigment pattern were visible in the specimen from Bahia Honda. Sixth thoracic somite with broad median, rectangular dark patch and smaller lateral patch on each side. Seventh thoracic somite with posteromedian black spot. First abdominal somite with median rectangular dark patch, fifth abdominal somite with smaller median spot. Each abdominal somite with lateral dark spot on each side. Telson with two anterior black spots between anterior submedian and median carina. Dorsal depression of merus of claw pink with proximal and distal dark spot.

Remarks. — This species can be distinguished by the large numbers of dorsal spinules on the telson, the laterally produced ocular scales, and the presence of a lateral denticle on the telson.

The median carina of the telson in *G. festae* is flanked laterally by a line of two to three tubercles on each side; the anchor is a coronet of spinules. In the larger males the spined accessory median carinae are obliterated by the inflated median carina; in all specimens the dorsal tubercles are still visible. The knob is armed with four to five tubercles or spinules. The dorsal spinules of the submedian carinae are usually arranged in two longitudinal rows. This is the only American species of *Gonodactylus* with a distinct lateral denticle on the telson; the denticle was illustrated by Schmitt (1940, figure 32c) but the legend to his figure is erroneous in placing the denticle on the carapace. Spination patterns of the telson are summarized in Table 5.

The anterolateral angles of the rostral plate in *G. festae* are sharp but never spiniform as in *G. bahiahondensis*. As pointed out by Schmitt (1940), the ocular scales are strongly produced laterally, giving them an angled appearance; the scales are more acutely angled laterally than in any other American species.

Gonodactylus bahiahondensis Schmitt, 1940

Gonodactylus bahiahondensis Schmitt, 1940, p. 217, fig. 31.

Range. — Eastern Pacific region, from scattered localities between Port Parker, Costa Rica, and Cape San Francisco, Ecuador.

Material examined. — Fifteen specimens from six stations:

Costa Rica

Port Parker; in coral; 16-17 January 1938;

one male, three females.

Port Parker; 12-23 January 1938; three females.

Port Culebra; coral; 24-31 January 1938; three males, one female (in two lots).

Jasper Island; coral; 22-25 February 1938; one male.

Uvita Bay; coral; two females.

Panama

Bahia Honda; under stone, low tide; 13-19 March 1938; one female.

Measurements. — Males, TL 18-36 mm; females, TL 22-41 mm. Abdominal Width-Carapace Length Indices are summarized in Table 4.

Color. — Faded in most specimens. One specimen was marked with three dark bands on the carapace, the middle one appearing as two submedian dark bars on either side of the midline. Each of the abdominal somites was marked with dark spots arranged more or less in bands. Some specimens had four anterior black spots on the telson. In general, all of the males had a darker background color than the females.

Remarks. — This species can be distinguished readily from the other American species of *Gonodactylus* with dorsal tubercles on the telson by the spiniform anterolateral angles on the rostral plate, the long accessory median carinae, each terminating in a posterior spine, and the presence of not more than two tubercles or spinules on the knob. As in both *G. lalibertadensis* and *G. stanschi*, the accessory median carinae do not fuse posteriorly to form an anchor. As pointed out by Schmitt (1940), the ocular scales are not produced laterally but are inclined anteriorly.

In large males the anterior portions of the accessory median carinae fuse with the inflated median carina, so that the accessory medians appear to be very short; in these specimens the dorsal tubercles of the telson are much smaller than in young specimens. The accessory medians may also disappear anteriorly in large females.

Patterns of dorsal spinulation of the telson are summarized in Table 5.

Gonodactylus lalibertadensis Schmitt, 1940

Gonodactylus festae lalibertadensis Schmitt, 1940, p. 223, fig. 33.

Range. — Eastern Pacific region, where it was known previously only from the type-locality, La Libertad, Santa Elena Bay, Ecuador. The present material extends the range northward to Port Culebra and Uvita Bay, Costa Rica.

Material examined. — Two specimens from two stations:

Costa Rica

Port Culebra; in coral; 24-31 January 1938; one female.

Uvita Bay; in coral; 2-4 March 1938; one female.

Measurements. — Females only examined, TL 27-37 mm. Abdominal Width-Carapace Length Indices are summarized in Table 4.

Color. — The specimen from Uvita Bay is covered with small, black chromatophores in no particular pattern; there are traces of two anterolateral black spots on the telson, each set on one of the anterior tubercles.

Remarks.—*Gonodactylus lalibertadensis* was originally described as a subspecies of *G. festae;* Schmitt pointed out that it resembled both that species and *G. bahiahondensis* in some features. The two specimens taken by the *Zaca* can be distinguished from representatives of both of those species, so it is recognized here as a distinct species.

Gonodactylus lalibertadensis closely resembles *G. bahiahondensis* and differs from *G. festae* in having long accessory median carinae which extend anteriorly to the base of the median carina; these carinae are always armed posteriorly and do not fuse posteriorly under the apex of the median carina to form an anchor. It differs from *G. bahiahondensis* and resembles *G. festae* in that the ocular scales are produced laterally, the anterolateral angles of the rostral plate are acute but not spiniform, the knob is armed with at least four spinules, and the tubercles of the submedian carinae usually are set in two distinct rows; each of the two specimens reported here have but two dorsal tubercles on the submedian teeth, both situated in a dorsal depression in one row. The overall spinulation pattern of the telson is summarized in Table 5.

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5

On the Resemblance of the Young of the Fishes *Platax pinnatus* and *Plectorhynchus chaetodontoides* to Flatworms and Nudibranchs

JOHN E. RANDALL[1] AND ALAN R. EMERY[2]

(Figures 1-4)

Juveniles of the high-bodied ephippid fish *Platax pinnatus* are black with a bright orange border. An individual 16 mm in standard length was observed in Palau to lie on its side and undulate its expanded fins in such a manner that it closely resembled a turbellarian flatworm. The noxious surface secretions of polyclads are believed to make them distasteful to predators; the bright liveries of many of them no doubt serve as warning coloration. In mimicking such a polyclad, a fish probably enjoys relative freedom from predation.

The young of the pomadasyid fish *Plectorhynchus chaetodontoides,* which swims with head oriented downward and excessive body and fin movement, and is colored conspicuously and very differently from adults, also bears some resemblance to soft-bodied invertebrates, such as nudibranchs or turbellarians.

WHILE COLLECTING fishes with rotenone at a depth of 15 meters at the base of a coral reef off Malakal Harbor in the Palau Islands, a flat, soft-bodied animal, black with a bright orange border, was observed by the senior author free in the water about two meters off the bottom. Oriented in a horizontal plane, it was swimming slowly with an undulating movement among several small fishes affected by the poison. It was regarded initially as a turbellarian flatworm. On occasions during fish-collecting operations with ichthyocides, benthic turbellarian flatworms may be seen detached from the bottom swimming gracefully but ineffectually, with characteristic marginal body undulations. When taken in dip nets or jars with fishes, flatworms invariably fragment and lose all value as specimens. Thus, no attempt was made to collect this one.

A few minutes later at the same station, the junior author was attracted to a similar "flatworm" (which may have been the same individual), undulating slowly on the bottom. When approached, it fluttered slowly away. Only after several seconds of close observation were the animal's eyes, mouth, and fins noticed, and it was realized that the "flatworm" was a fish. It had a compressed body and had lain on its side; the swimming undulations were carried out

principally by its expansive dorsal and anal fins. The fish was suffering some affects of the rotenone and was easily collected; it died soon after it was placed in a jar with other fishes.

The specimen (figure 1), now deposited in the Bernice P. Bishop Museum, Honolulu, measures 16 mm in standard length. We identify it as the ephippid *Platax pinnatus* (Linnaeus). The fin-ray counts (D V,38; A III,26; P_1 19) were useful in arriving at the identification. Also important was the reporting by earlier authors (Fowler and Bean, 1929; Weber and de Beaufort, 1936) of larger juveniles of *pinnatus* with a median orange band or broken line on the nape—evidently the last vestige of the peripheral orange band. The 140-mm specimen from Palau illustrated as figure 2 is an example of this intermediate coloration. It had several dots of orange-red medially along its forehead in life.

Bleeker (1860) described a 54-mm blackish fish from Ambon as *Platax melanosoma*. It is clear from the illustration of this form in his *Atlas Ichthyologique* (1877, pl. 380, fig. 4) that *melanosoma* is the young of *pinnatus*. The median orange-red band on the head and a partial orange-red border on the dorsal and anal fins are readily seen on the illustration.

Adults of *P. pinnatus* lose all trace of the orange markings. A specimen from Palau, 224 mm in standard length (BPBM 9003), was grayish silver in life with a faint dark bar through the eye and another from the origin of the dorsal fin to the pelvic fins; the dorsal and anal fins

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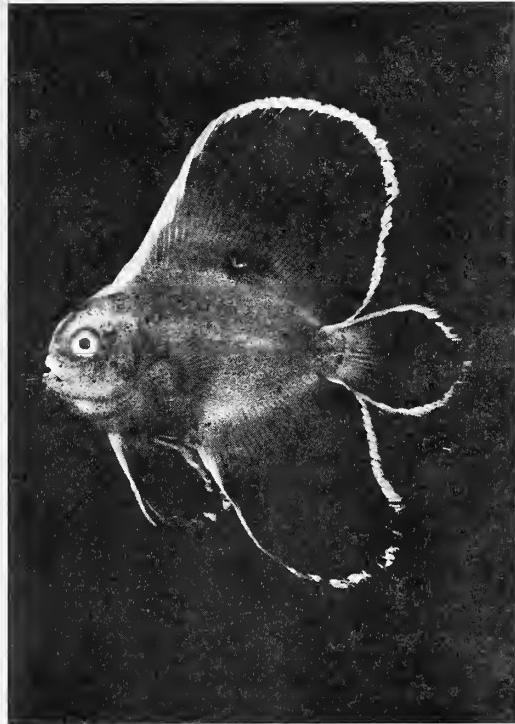
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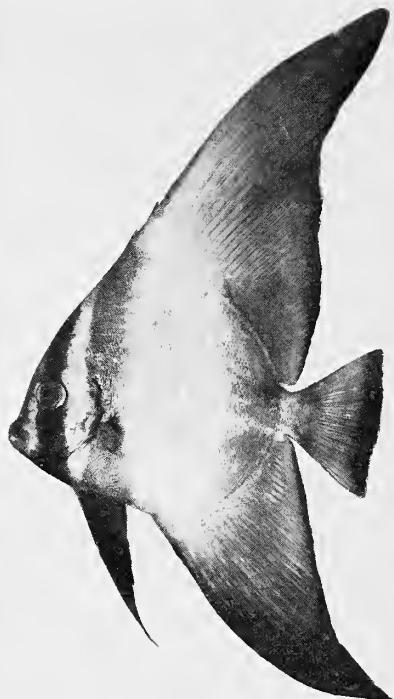
FIGURE 1. *Platax pinnatus,* 16 mm standard length, BPBM 9456, collected in the Palau Islands on April 16, 1970. The pale mark at the base of the dorsal fin surrounded by black is a wound—probably inflicted during collecting. In life the body and fins were entirely black except for the bright orange border.

FIGURE 2. *Platax pinnatus,* 140 mm standard length, BPBM 9530, collected in the Palau Islands on April 12, 1970.

were yellowish; the caudal was yellowish with a broad blackish posterior border; the pectorals were yellow, dusky basally; the pelvics were mainly blackish.

Like other *Platax,* the exceedingly high dorsal and anal fins become relatively shorter with increasing size. *P. pinnatus* is distinctive in developing a concavity in the dorsal profile of the snout as an adult.

Judging from the configuration of the 16-mm mimic, its lack of scales, and small slender canine teeth (tricuspid in larger juveniles and adults), it is not fully transformed from the late postlarval stage.

In its guise as a flatworm, juvenile *Platax pinnatus* may at least partially avoid predation. Turbellaria are seldom eaten by other animals, as their surface secretions appear to be distasteful, if not actually toxic (Hyman, 1951). Many of the polyclads, particularly in warm seas, are strikingly colored. Undoubtedly these bright and contrasting hues serve as warning coloration.

Polyclads often have a brightly colored border, and at least two are known which are dark with a broad orange margin or submarginal zone. One is *Callioplana marginata* (Stimpson) from Japan and the other, *Pseudoceros affinis* (Kelaart), is known from Ceylon to the Hawaiian Islands (Hyman, 1960). The latter has been illustrated in color (Collingwood, 1876; Stummer-Traunfels, 1933).

Some nudibranchs also have similar color patterns. One of the color phases of the Indo-Pacific *Dentrodoris nigra* (Stimpson), for example, is black with a scarlet border (personal communications, E. Alison Kay). Nudibranchs are known to produce noxious secretions, and some make second-hand use of coelenterate nematocysts.

In the genus *Platax,* the habit of resembling some other organism or object of little interest to predators is not restricted to the species *pinnatus.* Juvenile *Platax orbicularis* (Forsskål) [for an illustration, see Taylor, 1964] were observed in the Society Islands to drift on their sides with floating yellowish leaves of *Hibiscus tileaceus* Linnaeus, which they resembled most closely (Randall and Randall, 1960). Other comparable observations have been made (Willey, 1904; Mortensen, 1917; Uchida, 1951).

The habit of mimicking a flatworm or nudibranch is not unique to *Platax pinnatus.* The young of the pomadasyid fish *Plectorhynchus chaetodontoides* Lacépède (figure 3) was observed in Palau by the senior author to swim in a very unusual manner, with head oriented downward, excessive body flexure, and more flopping of fins than would seem necessary for normal progression. The dorsal and caudal fins are proportionately longer in the juveniles than adults. The color is orangish brown with large dark-edged white areas—very different from adults which are silvery gray with numerous small black spots (a subadult is illustrated as figure 4). The conspicuous color and mode of swimming of the juvenile *P. chaetodontoides* served to draw attention to itself. Although the fish did not present as convincing a resemblance to a flatworm or nudibranch as *Platax pinnatus,* the first sighting gave the distinct impression of a soft-bodied invertebrate of these groups.

Juveniles of other species of *Plectorhynchus* may also be strikingly colored and swim in a similar peculiar manner. It has been stated that they seem to flutter through the water like the dancing or cavorting of clowns (Herald, 1961). Their movements are decidedly unlike those expected of fishes.

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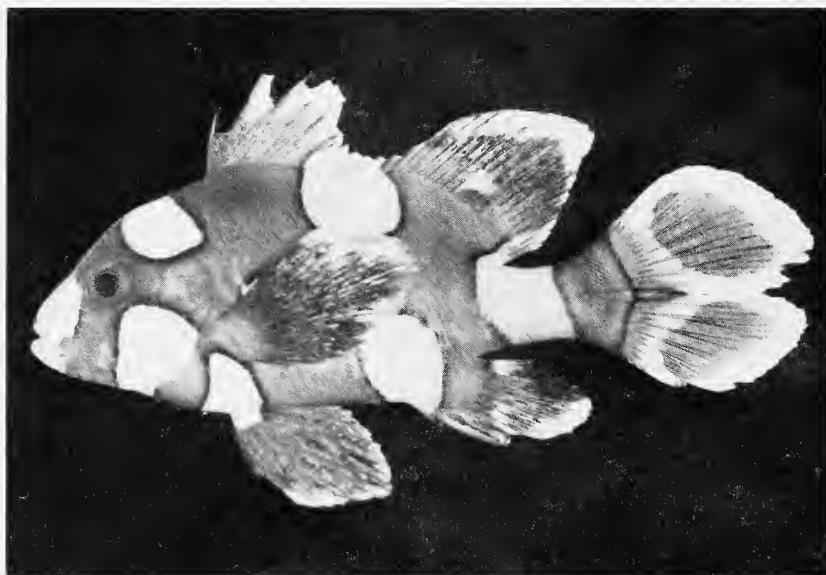
FIGURE 3. *Plectorhynchus chaetodontoides,* 37 mm standard length, BPBM 9240, collected in the Palau Islands on October 5, 1966.
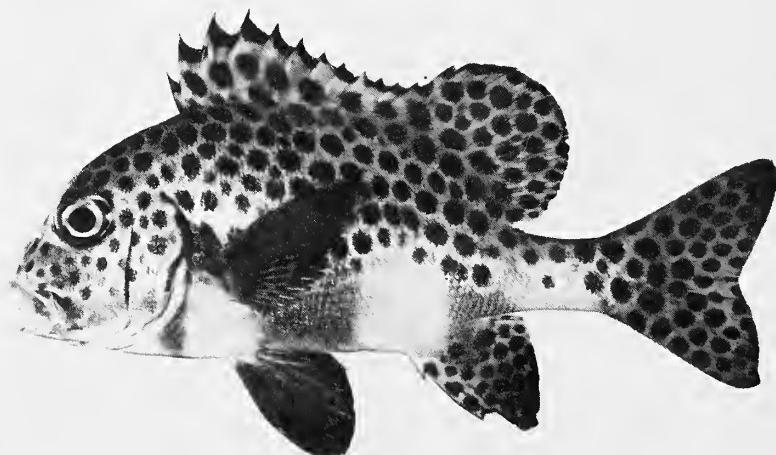
FIGURE 4. *Plectorhynchus chaetodontoides,* 185 mm standard length, BPBM 9480, collected in the Palau Islands on April 15, 1970.

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6

Chromosome Numbers of Heliconiine Butterflies from Trinidad, West Indies (Lepidoptera, Nymphalidae)

(Figures 1-4; Tables 1-2)

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John R. G. Turner
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Haploid numbers are given for all 14 of the Heliconiine butterflies found in Trinidad. Eleven have been examined in other parts of their range, and show no geographical variation in chromosome number. Three species show variation within or between individuals. *Heliconius melpomene* and *H. erato* vary so much in their color pattern that they are usually split into several species; there is no accompanying variation in chromosome number. Classification of the subfamily by karyotype matches well with the conventional classification. Most of the genera and subgenera have n = 28 to n = 31, which is typical of butterflies, but the very numerous subgenus *Heliconius* (*Heliconius*) has, with a few exceptions, n = 21. The chromosome numbers of all species in the subfamily examined by these and other authors are tabulated. The three previously unreported species are *Philaethria dido, Heliconius ethilla,* and *H. ricini* (all n = 21).

INTRODUCTION

MUCH IS NOW KNOWN about the physiology, ecology, genetics, behavior, and geographical distribution of the butterflies belonging to the predominantly Neotropical subfamily Heliconiinae; most of the recent papers have been published in this journal. This paper reports the chromosome numbers of all the 14 species found in Trinidad, West Indies. Four of these species, *Philaethria dido, Heliconius ricini, H. ethilla,* and *H. wallacei* have not been examined before; the remaining ten Trinidadian species all have been examined, in other parts of their range by de Lesse (1967) and by Maeki and Remington (1961). (Since this paper went to press de Lesse (1970b) has examined *H. wallacei* from Guyane, formerly French Guiana).

METHODS

Testes were taken from adult males and ovaries with mature eggs from old adult females. They were fixed in Carnoy's fluid (6:3:1), embedded in paraffin via butyl alcohol and sectioned at a thickness of 10 μ (testes) or 15 μ (ovaries). The preparations were stained by the Feulgen method.

RESULTS

Haploid chromosome numbers for all the Trinidadian species are given in Table 1, with de Lesse's data from other parts of Spanish America, Maeki and Remington's from México, and Emmel's data from Costa Rica for comparison. The 11 species investigated both in Trinidad and in other parts of their range show no geographical variation of chromosome number, but *H. aliphera, H. isabella,* and *H. doris* show variation within or between individuals. Figures 1-4 show the chromosomes of the four species not previously described.

The use of the name *H. ethilla* (Godart) requires some explanation; in most other recent papers on this group, the species is known as *H. numata* (Cramer) (see for example Beebe, Crane, and Fleming, 1960, and Turner, 1968a). The change of name to *ethilla* results from the revision by Emsley (1965), and the extensive revision of this section of the genus by Brown (1972). De Lesse (1967) reports the chromosome number of a species from Colombia which he calls *H. ethilla;* Dr. K. S. Brown, Jr. (personal communication) has kindly identified this as *H. hecale melicerta* Bates.

All other names quoted are used in the same sense as in the many papers on this group appear-

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[1] On leave of absence from the State University of New York at Stony Brook.

ing in *Zoologica,* and illustrated by Beebe *et al.* (1960) and Emsley (1963) (see Turner, 1967, for a note on the complicated tangle over the names *doris, erato,* and *vesta*). Authors are cited in Table 2.

Discussion

The lack of geographical variation in the chromosome numbers of the species *Heliconius erato* and *H. melpomene* is worth noting because both show such remarkable geographical variation of the wing pattern (illustrated in color by Turner, 1970, 1971) that most taxonomists have split them into several species (Neustetter, 1929). Table 1 shows that four distinct subspecies of *melpomene* (*H. m. melpomene* from Trinidad and Colombia, *H. m. thelxiope* from Guyane, *H. m. plesseni* from Ecuador and *H. m. rosina* from Costa Rica), representing four very different color patterns, all have the same chromosome number. Similarly the same chromosome number is found in five distinctly marked subspecies of *H. erato* (*H. e. hydara* or *guarica* from Trinidad, Guyane, and Colombia; *H. e. erato* from Guyane; *H. e. venustus* from Bolivia; *H. e. phyllis* from Argentina; and *H. e. petiverana* from México and Guatemala). Thus the evolution of elaborate differences of pattern in these two species has

not been accompanied by any change in chromosome numbers (see Turner, 1971, for a discussion).

In Table 2, the subfamily is classified according to the haploid chromosome numbers. Several interesting facts emerge. The subgenus *Heliconius* (*Heliconius*) consistently has a haploid number of 21, with the exception of *H. sapho* and *H. congener* (at one time thought to be conspecific), which both have exceptionally high numbers. The other subgenera within the genus *Heliconius* have chromosome numbers greater than 21. *Eueides* has a haploid number of 31 to 33, and *Laparus* (represented by one species, *doris*) and the unnamed subgenus containing *H. aoede* have haploid numbers between 23 and 27. These last two subgenera were tentatively separated from *Heliconius* (*Heliconius*) by Turner (1968b), by the morphology of their pupae.

Four of the other genera, *Agraulis, Dione, Dryas,* and *Dryadula,* frequently placed in one genus before Michener's revision of the group (Michener, 1942), resemble *H. (Eueides)* with n = 31. *Philaethria* is like *Heliconius (Heliconius)* in having n = 21. *Podotricha* has one species with a number slightly below 31 (i.e. 28-29) and another with an exceptionally low number (8-10), which approaches the lowest

TABLE 1. HAPLOID CHROMOSOME NUMBERS OF THE TRINIDADIAN HELICONIINE BUTTERFLIES (NEW DATA), WITH DATA FROM OTHER AREAS (DE LESSE, 1967, 1970a, 1970b; MAEKI AND REMINGTON, 1961; EMMEL, 1969) FOR COMPARISON

Species	Trinidad ♂	Trinidad ♀	Guyane	Colombia	Bolivia* or Ecuador†	Argentina	México** or Guatemala†† or Costa Rica‡‡
Agraulis vanillae	31	—	—	—	—	31	—
Dione juno	31	—	—	—	31*†	31	31**
Dryas iulia	31	—	31	31	—	31	31**
Dryadula phaetusa	—	31	—	—	—	31	—
Philaethria dido	21	—	—	—	—	—	—
Heliconius isabella	30– 31	—	—	31 —	—	—	31**
H. aliphera	—	30, 31	—	—	—	31	—
H. doris	—	26–27		24–25, 26, 27	—	—	—
H. ethilla	21	21	—	—	—	—	—
H. melpomene	21	21	21‡	21	21†	—	21‡‡
H. erato	21	21	21§	21	21*	21	21**††
H. sara	21	21	21	21	21†	—	—
H. ricini	21	21	—	—	—	—	—
H. wallacei	21	—	21	—	—	—	—

‡ Lower-Amazonian subspecies, with a slight hybrid admixture from the Colombian-Trinidadian subspecies.

§ Both the lower-Amazonian and Colombian-Trinidadian subspecies.

Numbers separated by a hyphen are found in the same individual; numbers separated by a comma are found in different individuals.

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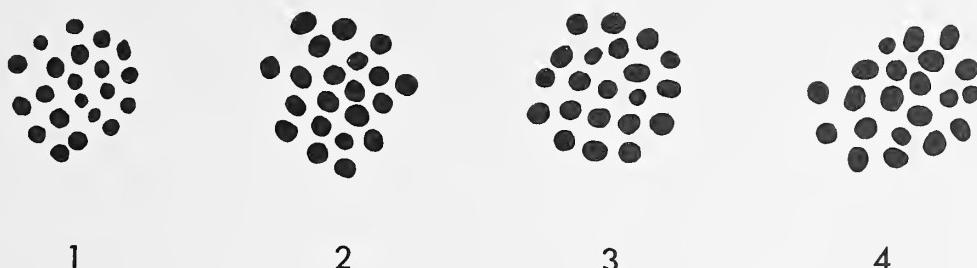
FIGURE 1. *Philaethria dido*. Secondary spermatocyte metaphase.

FIGURES 2–4. Primary spermatocyte metaphases.

FIGURE 2. *Heliconius ethilla*.

FIGURE 3. *H. ricini*.

FIGURE 4. *H. wallacei*. Magnification on all figures × 4000.

haploid number known in the Lepidoptera (*Erebia tyndarus* has 8—Lorkovic, 1949; *Agathymus aryxna* has 5; and three other *Agathymus* have 9—Freeman, 1970). The haploid number 31 is very common in the Nymphalidae, of which the Heliconiines are members, and is the commonest number in the Lepidoptera (Suomalainen, 1969).

The chromosome numbers show themselves to be very much in accord with the taxonomy of the subfamily as determined by the morphology of the adults and pupae (Emsley, 1963; Turner, 1968b; Brown and Mielke, 1971). Most of the genera and subgenera have retained the haploid number of the majority of Lepidoptera (n = 29-32), but the widespread and numerous group *Heliconius* (*Heliconius*) has stabilized, as Suomalainen (1969) points out, round a new number of n = 21. It is interesting that the species with the typical Nymphalid number of 31 include those which have deviated least from the Nymphalids in wing-shape and coloring.

TABLE 2. CLASSIFICATION OF THE HELICONIINAE BY CHROMOSOME NUMBERS IN RELATION TO THE "NORMAL" NUMBER FOR LEPIDOPTERA (n = 29 − 32)
(DATA FROM PRESENT PAPER, MAEKI AND REMINGTON (1961), AND DE LESSE (1967, 1970a, 1970b)

HIGH CHROMOSOME NUMBER		n = 21	*Philaethria dido* (Clerck)
n = 56	*Heliconius* (*Heliconius*) *sapho* (Drury)		*Heliconius* (*Heliconius*) *melpomene* (L.)
n = 33	*H.* (*H.*) *congener* Weymer		*H.* (*H.*) *timareta* (Hewitson)
n = 32–33	*H.* (*Eueides*) *lybia* (Fabricius)		*H.* (*H.*) *elevatus* Nöldner
			H. (*H.*) *cydno* (Doubleday)
NORMAL CHROMOSOME NUMBER			*H.* (*H.*) *ethilla* (Godart)
n = 31	*Agraulis vanillae* (L.)		*H.* (*H.*) *hecale* (Fabricius)*
	Dione juno (Cramer)		*H.* (*H.*) *ismenius* (Latreille)†
	Dryas iulia (Fabricius)		*H.* (*H.*) *erato* (L.)
	Dryadula phaetusa (L.)		*H.* (*H.*) *ricini* (L.)
	Heliconius (*Eueides*) *aliphera* (Godart)		*H.* (*H.*) *hortense* (Guérin-Meneville)
	H. (*E.*) *isabella* (Cramer)		*H.* (*H.*) *charitonia* (L.)
n = 28–29	*Podotricha telesiphe* (Hewitson)		*H.* (*H.*) *sara* (Fabricius)
			H. (*H.*) *clysonymus* Latreille
LOW CHROMOSOME NUMBER			*H.* (*H.*) *atthis* (Doubleday)
			H. (*H.*) *telesiphe* (Doubleday)
n = 24–27	*Heliconius* (*Laparus*) *doris* (L.)		*H.* (*H.*) *wallacei* Reakirt
n = 23	*Heliconius* (−) *aoede* (Hübner)	n = 8–10	*Podotricha euchroia* (Doubleday)

* From Colombia; identified by de Lesse (1967) as *H. ethilla* (see text).

† From México; identified by de Lesse (1970a) as *H. numata telchinia,* and attributed to the species *ismenius* from the studies of K. S. Brown, Jr. (personal communication).

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Summary

1. Haploid chromosome numbers are given for all 14 species of Heliconiine butterflies found in Trinidad. Eleven of these have also been investigated in other parts of their range, and none shows any geographical variation in chromosome number.

2. The great geographical variation in color of *Heliconius melpomene* and *H. erato* is not accompanied by any variation in chromosome numbers.

3. Most of the genera and subgenera have numbers between 28 and 31, which are typical of Lepidoptera, but the very successful subgenus *Heliconius* (*Heliconius*) has, with a few exceptions, a haploid number of n = 21.

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The Genetics of Some Polymorphic Forms of the Butterflies *Heliconius melpomene* (Linnaeus) and *H. erato* (Linnaeus).

II. The Hybridization of Subspecies of *H. melpomene* from Surinam and Trinidad.[1,2]

(Plate-figures 1-37; Text-figures 1-4; Tables 1-10)

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To investigate the genetics of mimetic patterns in *H. melpomene,* which mimics its own relatives in the genus *Heliconius,* crosses were performed between geographical races from Trinidad and central Suriname (South America). The differences between the color patterns, so distinct as to be classed as separate species by some authors, are controlled by four major loci (designated *B, D, N,* and *F*), all showing complete dominance except for *N.* The only linkage is between *B* and *D,* with crossovers less than 16 percent. Some of the F_2 hybrids showed patterns not found in the parental subspecies, but known in other races or in related species. These were in part controlled by the *N* locus. There is evidence of other loci, in addition to the four major ones, affecting the pattern. The race from eastern Amazonia differs from that in central Suriname by an allele at the *D* locus. In northern Suriname, these three races hybridize naturally, producing a very elaborate polymorphism. Previous workers have bred butterflies from this polymorphic area and have therefore only partly solved the genetics of the race differences. The present study, tabulating 54 crosses (over 1,000 butterflies), has probably detected most of the major genes differentiating the three subspecies.

INTRODUCTION

THE NEOTROPICAL BUTTERFLY *Heliconius melpomene* is interesting because of its close mimicry of its relative *Heliconius erato* (illustrated by Turner, 1970), and because it exhibits elaborate polymorphisms in certain parts of its range. Research into this Müllerian mimicry and polymorphism is likely to be particularly profitable, as the New York Zoological Society has conducted intensive research into many aspects of the biology of *Heliconius* and related genera (for a review of certain aspects, see Turner, 1971a), giving exceptional opportunities for integrated research.

This paper describes a thorough investigation of the genetics of the polymorphism of *melpomene* in the Guianas. The main aim was to solve certain problems posed by previous breeding experiments with this species (Turner and Crane, 1962; Sheppard, 1963); it is thought that most of the genetic factors have now been identified. Sheppard (1963) found that in this species, different genes can produce identical phenotypes; for this reason the new broods will be analyzed first, and the results will then be compared with those of the previous workers.

Turner and Crane (1962) and Sheppard (1963) obtained their stocks from the monomorphic populations of the species in Trinidad (Text-fig. 1c) and from Moengo in Surinam (Text-fig. 2) where there is a polymorphic popu-

lation consisting mostly of butterflies like those in Trinidad, with a small percentage of "mutant" forms; frequencies are given by Sheppard (1963). The present broods are different from these in one most important way: the Surinam stock was obtained at Brokopondo (Text-fig. 2), where *melpomene* has a population consisting mostly of butterflies with the pattern shown in text-fig. 1b, and very unlike individuals from Trinidad. The stocks were thus started with lines differing simultaneously at several loci.

It will be shown elsewhere (Turner, 1971b) that the population at Brokopondo is on the edge of a subspecies of *melpomene* (*H. melpomene meriana* Turner), found in the interior of the Guianas. The present breeding experiments are tantamount to hybridization of two subspecies of *melpomene*, although, rather conveniently, the slight degree of polymorphism of the Brokopondo population resulted in a few segregating broods produced by wild-caught females. This considerably speeded the work.

This paper replaces the paper originally projected as the second in this series (and cited by Turner and Crane, 1962), as the matter to be discussed (the inheritance of various yellow markings) is much more clearly understood from the new broods reported here.

METHODS

The parent butterflies which founded the Surinam stock were captured September 22, 1964, in disturbed rain-forest near Brokopondo, which lies almost as far into the interior of Surinam as can be reached by road. They were kept overnight in a portable gauze cage, fed forcibly on sucrose solution, and flown next day to Trinidad. Trinidadian parent butterflies were reared from early stages found in northern Trinidad.

The methods of mating and rearing the insects in Trinidad have already been described (Turner and Crane, 1962). In December 1964, the larvae (with leaves of the food plant) and the pupae were packed in capped, plastic tubes, and the imagines were placed in paper photo-negative envelopes, with a wad of cotton-wool soaked in sucrose solution. The whole stock was placed in an insulated picnic hamper, and carried to Britain in the passenger cabin of a jet airliner. Despite the decompression and, on arrival, a train journey of several hours through mid-winter Britain, the stock reached Liverpool with all the insects alive.

In Liverpool the larvae were reared in plastic boxes, several to a box, or in cloth sleeves on food plants growing in greenhouses. Greenhouses were used for mating and for housing laying females. Butterflies were fed from dishes of honey mixed with water. Only four greenhouses were available, including one used for a group of mixed broods, which were maintained as a general reservoir of genes without full records. As a female requires a whole small greenhouse to fly in, we could keep only three fertile females at a time, and as mortality of adults and larvae was much higher than it had been in Trinidad, broods from Liverpool were smaller than those from Trinidad. Despite the less than optimum conditions, stocks were maintained until May 1965.

In Trinidad, *Passiflora laurifolia* and *P. serrato-digitata* were used as food plants; in Liverpool, many of the larvae were reared on the horticultural hybrid *P. allardi,* which they readily accepted.

Specimens are preserved in translucent envelopes (see Turner and Crane, 1962) and stored in a filing system. With the large number of cages used in Trinidad, breeding strategy was

TEXT-FIGURE 1. Three forms of *Heliconius melpomene:* (a) the Amazonian *H. m. thelxiope* (Hübner); (b) the Surinamian *H. m. meriana* Turner; (c) the Trinidadian and Venezuelan *H. m. melpomene* (Linnaeus). About 0.8 times natural size.

comparatively easy, and almost every butterfly has been kept. In Liverpool it was necessary to release a large number of butterflies into the greenhouse containing the general stock, and because of this, and the loss of butterflies which died naturally behind the elaborate fittings in the greenhouse, not all the specimens hatched in Liverpool are preserved.

The results are shown in Tables 1-7. A "?" indicates a wild butterfly, mated to one of the Surinam females, and never seen; parental butterflies are described by their brood of origin (with "Surinam" and "Trinidad" for wild butterflies, and "Liverpool" for butterflies from the general gene-reservoir already mentioned) and phenotype; the mating numbers merely refer to the original brood books and are not used in the text; a small superscript letter (e.g.[a]) identifies a butterfly which was the parent of more than one brood; § indicates that several males were placed with one female, and that the butterfly listed is the most probable mate, in view of the progeny obtained. Phenotype formulae are described in the next section; brackets indicate a doubtful phenotype.

The system of individual rearing and labeling used in Trinidad resulted in extremely few errors. Only two such mistakes appeared: on S[F]Dr butterfly in brood 17 and a W[F]dr in brood 11; clearly the labels had simply been reversed,

and the butterflies have been placed in their correct brood in the tables.

MAJOR GENES

Inheritance of dennis and radiate

The pattern known as "dennis" (Dr) consists of extensive red patches covering the basal half of the forewings, and a small red triangle at the base of the hindwings. The radiate pattern (DR) is the same as dennis, but has in addition about six red rays between the veins on the hindwings. A butterfly lacking these red marks is called plain (dr). The plates and text-fig. 1 show examples.

Trinidadian butterflies are always plain; all the butterflies from Brokopondo were dennis, but one female produced a brood that segregated dennis and radiate in equal numbers (Table 1, brood 3).

Dennis is produced by a single gene dominant to plain; the F_1 hybrids between Surinam (dennis) and Trinidad (plain) are all dennis (Table 1, broods 5-7), the F_2 segregates 64 dennis to 27 plain (Table 4, total), and the backcross to Trinidad segregates 64 dennis to 69 plain (Table 3, total). Radiate is dominant to plain, and likewise dominant or epistatic to dennis. Thus Surinam radiate butterflies mated to plain Trinidad butterflies yielded broods consisting equally of radiate and dennis (ratio 17 DR to 21 Dr),

TEXT-FIGURE 2. The Guianas, showing Moengo and Brokopondo, where polymorphic forms of *melpomene* have been obtained for breeding experiments.

TABLE 1. OFFSPRING OF WILD SURINAM FEMALES, AND F_1 SURINAM × TRINIDAD HYBRIDS

		PARENTS			RADIATE (DR)		DENNIS (Dr)				
BROOD	MATING	♀	♂		Y^F	TS^F	Y^F	TY^F	S^F	TS^F	Total
1	M5	Surinam	Surinam	♂	0	0	10	3	5	7	47
		TY^FDr	?	♀	0	0	6	5	6	5	
2	M6	Surinam	Surinam	♂	0	0	17	0	11	0	57
		S^FDr	?	♀	0	0	15	0	14	0	
3	M7	Surinam[a]	Surinam	♂	26	0	20	0	0	0	80
		Y^FDr	?	♀	16	0	18	0	0	0	
4	M9	Surinam	Surinam	♂	0	0	16	0	0	0	23
		Y^FDr	?	♀	0	0	7	0	0	0	
5	M13	Trinidad	Surinam	♂	0	0	0	0	0	26	56
		W^fdr	Y^FDr	♀	0	0	0	0	0	30	
6	M14	Trinidad	Surinam	♂	0	0	0	0	0	22	51
		W^fdr	Y^FDr	♀	0	0	0	0	0	29	
7	M16	Trinidad	Surinam[d]	♂	0	0	0	0	0	9	21
		W^fdr	Y^FDr	♀	0	0	0	0	0	12	
8	M22	3	Trinidad[b]	♂	0	4	0	0	0	4	20
		Y^FDR	W^fdr	♀	0	4	0	0	0	8	
9	M23	3	Trinidad	♂	0	6	0	0	0	4	19
		Y^FDR	W^fdr	♀	0	3	0	0	0	6	

showing that the radiate pattern is dominant to plain (which is not expressed in the hybrid), and also to dennis (which must have been carried, but not expressed, by the radiate parent) (Table 1, broods 8-9). Strictly we should say that radiate is "not recessive," rather than "dominant" as a radiate homozygote has not for certain been produced; brood 50 (Table 7) is a cross between two radiate butterflies, but the 6 radiate offspring are too few to contain, with reasonable probability, a homozygote. But it is likely that the radiate homozygote is in fact like the heterozygote, and the gene completely dominant, as the subspecies *thelxiope* from Pará is monomorphic, and so presumably homozygous, for this pattern.

Allelism of dennis and radiate

As the radiate pattern contains the dennis pattern, it seems fairly safe to assume that radiate and dennis are alleles at the same locus. However they might well be produced by two independent loci, which both exploited the same developmental pathway. Broods 8, 18, 21, and 34 show that they are in fact alleles at a single locus. A Surinam radiate butterfly was mated to a plain from Trinidad. The progeny (Table 1, brood 8) show that the radiate parent was carrying dennis but not plain. Therefore if radiate and dennis were at different loci, with radiate epistatic to dennis, the radiate progeny in brood 8 should be carrying both dennis and plain; on mating to a plain butterfly, they should give

radiate, dennis, and plain in the ratio 2 : 1 : 1. On the other hand, if radiate and dennis are allelic, the radiate progeny in brood 8 can carry only radiate and plain, and on crossing to a plain butterfly will give radiate and plain in the ratio 1 : 1, but no dennis.

Two of the radiate progeny, mated to plain butterflies produced, in broods 18, 21, and 34 (Tables 3 and 5), 17 butterflies which were either radiate or plain; there were no dennis. The appropriate statistical test is to calculate the probability that no dennis butterflies will appear in a brood of 17 individuals when one quarter of the brood are expected to be dennis. The test is one-sided; for if the whole brood was by chance dennis, no statistical test would be needed. By the binomial theorem, the probability of this happening is $(0.75)^{17} = 0.0075$, which is highly significant. It is reasonable, therefore, to conclude that radiate and dennis are not carried at separate loci, but are allelic.

The above test does not of course rule out the possibility that radiate and dennis are at two loci which are linked rather closely, but as they are certainly on the same chromosome, it is simpler to regard them as alleles. The patterns radiate, dennis, and plain can be thought of as controlled by three alleles D^R, D, and d. An alternative hypothesis is that the patterns are controlled by two closely linked loci, one (D) producing the dennis pattern, the other (R) adding the rays. The patterns would then be con-

TEXT-FIGURE 3. The three main forms of the Ecuadorian species *Heliconius timareta* (Hewitson). About ¾ times natural size.

trolled by three chromosomes *DR*, *Dr*, and *dr*. This theory is supported by the closely related species *Heliconius timareta* from the eastern slopes of the Andes in Ecuador, one of whose three main forms has rays without the dennis pattern (Text-fig. 3). If dennis and ray are indeed closely linked loci, then the recombination value is very low, as no individual showing ray without dennis has ever been found among the thousands of specimens exported from the polymorphic populations of Guyane (Joicey and Kaye, 1917).

Inheritance of color of bands

The band on the forewing may be of the following types, designated by letters in the tables:

Y or "yellow". A group of firm pale yellow marks outside and inside the cell; the scales within the marks are entirely yellow and not mixed with black (Plate-figs. 1-4).

TY or "thin red with yellow". Like "yellow," but having a thin red band round the outside of the outermost yellow marks; the width of the red varies, but is usually no more than a series of red edgings to the outermost and most anterior yellow spots (Plate-figs. 5-9).

S or "dusky yellow". A group of pale yellow green marks in the same positions as those of Y; the marks tend to be smaller than in Y, and the yellow patch in the cell is often reduced or absent. The yellow green color is produced by a mixture of black and yellow scales (Plate-figs. 10-13).

TS or "thin red with dusky yellow". Like S, but with a thin red band exterior to the yellow marks; this red band varies in width, but is normally much wider than it is in TY, being a definite bar of red curving through the whole length of the area occupied by the band (Plate-figs. 14-19).

TABLE 2. FIRST GENERATION BACKCROSSES TO SURINAM STOCK
(See Also Brood 46 in Table 7)

BROOD	MATING	PARENTS ♀	♂		RADIATE (DR) Y^F	TY^F	S^F	TS^F	DENNIS (Dr) Y^F	TY^F	S^F	TS^F	Total
10	M36	6	3	♂	0	0	0	1	0	0	0	2	5
		TS^FDr	Y^FDR	♀	1	0	0	0	0	0	0	1	
11	M49	3	5^c	♂	0	0	2	2	3	8	6	2	42
		Y^FDR	TS^FDr	♀	0	6	1	0	1	3	6	2	
12	M50	7	Surinam^d	♂	0	0	0	0	3	3	1	1	13
		TS^FDr	Y^FDr	♀	0	0	0	0	1	1	2	1	
13	M51	5	3^e	♂	1	6	3	2	1	7	3	0	52
		TS^FDr	Y^FDR	♀	5	1	5	3	5	1	3	6	
14	M52	5	3^e	♂	5	0	1	0	1	2	3	2	25
		TS^FDr	Y^FDR	♀	1	2	2	3	0	2	1	0	
15	M60	Combination		♂	1	0	1	0	0	0	0	0	3
		of M51 and M52		♀	0	0	1	0	0	0	0	0	
		Total*			14	15	16	11	11	23	22	15	127*
		Total*				56				71			127*

* Excluding brood 12.

O or "absent band". The band is virtually absent, and is represented only by a few faint green-yellow marks in the region of the cell, and a red C-shaped mark near the posterior angle of the wing (Plate-fig. 20).

W or "wide red". A broad red band covering all the area in the region of the cell; in Trinidadian butterflies and in many of the hybrids, this is covered on the underside with white (sometimes yellow) scales (Plate-figs. 21-23).

The Trinidadian butterflies were always W; all Surinamian butterflies used as parents of F_1 and backcross generations were Y; two others were S and TY, and their offspring are not listed with F_1's or backcrosses.

Early in the experiments, it became obvious that band-color was controlled by more than one locus. The TY female from Surinam (mate unknown) produced a brood (Table 1, brood 1) of Y, TY, S, and TS in roughly equal numbers, suggesting that two loci are segregating. The S female from Surinam produced a brood (Table 1, brood 2) (apparently a backcross) equally of S and Y; an S × Y cross produced a similar backcross brood (table 7, brood 39). The F_1 Surinam × Trinidad hybrids (Y × W) are always TS (Table 1, broods 5-9). An S female from brood 2 mated to a W Trinidadian male, produced approximately equal numbers of TS and W offspring (Table 7, brood 41).

From these results, and bearing in mind the findings of Turner and Crane (1962) and Shep-

pard (1963), I formed the hypothesis about the inheritance of band-color illustrated in text-fig. 4. Two loci, B and N, are involved; the recessive allele b reduces the amount of red in the band, and the semi-dominant allele N^N reduces the amount of red and increases the amount of yellow. W butterflies from Trinidad are of the genotype BBN^BN^B; Y butterflies from Surinam are bbN^NN^N; the S phenotype is produced by the heterozygote N^NN^B, and the addition or subtraction of the red T mark from the S or the Y pattern is controlled by the substitution of B for b. The phenotypes of BBN^NN^N and bbN^BN^B butterflies (top right and bottom left of text-fig. 4) were uncertain, but I guessed that they would be TY and something similar to TS (not shown in text-fig. 4).

Broods which emerged after the formation of this hypothesis confirmed it, except in one detail, the phenotype of bbN^BN^B. This genotype has been produced in two matings between butterflies known to be BbN^BN^B (Table 6, broods 35 and 37), and turns out to be "absent-band" or O (Text-fig. 4; Plate-fig. 20).

Apart from this modification all test-crosses performed conform to the hypothesis. Thus Y should be homozygous; broods 40, 42, 44, and 48 which are Y × Y matings produced 80 Y butterflies in all, and no other phenotypes (the anomalous individual in brood 50 will be discussed later). An S × S mating should produce O, S, and Y; brood 47 (Table 7) has produced

TABLE 3. FIRST GENERATION BACKCROSSES TO TRINIDAD
(AND A SIMILAR BROOD)

BROOD	MATING	PARENTS ♀		♂		DENNIS (Dr) TS^F	TS^f	W^F	W^f	PLAIN (dr) TS^F	TS^f	W^F	W^f	Total
16	M21	Trinidad	1	♂		1	1	0	3	0	0	0	2	20
		W^{f}dr	TS^{f}Dr	♀		2	1	0	2	1	4	1	2	
17	M46	Trinidad	5^c	♂		3	3	4	7	3	6	3	3	61
		W^{f}dr	TS^{f}Dr	♀		3	4	3	6	4	2	4	3	
18	M58	Trinidad	8	♂		0	0	0	0	0	0	0	0	1*
		W^{f}dr	TS^{F}DR	♀		0	0	0	0	0	0	0	0	
19	M35	6	Trinidad^b	♂		4	0	0	0	1	4	0	3	23†
		TS^{F}Dr	W^{f}dr	♀		1	1	2	0	0	2	0	4	
20	M41	5	Trinidad	♂		1	1	1	3	1	2	0	3	28
		TS^{F}Dr	W^{f}dr	♀		3	0	2	2	1	2	4	2	
21	M56	8	Trinidad	♂		0	0	0	0	0	0	0	0	1‡
		TS^{F}DR	W^{f}dr	♀		0	0	0	0	0	0	0	0	
	Total					18	11	12	23	11	22	12	22	131
	Total					29		35		34*		35†		134*†‡
	Total					64				69*†				134*†‡

* 1 male, TSdr, not scored for F.

† Includes one Wdr butterfly, not scored for F or sex.

‡ 1 male, TS^{F}DR.
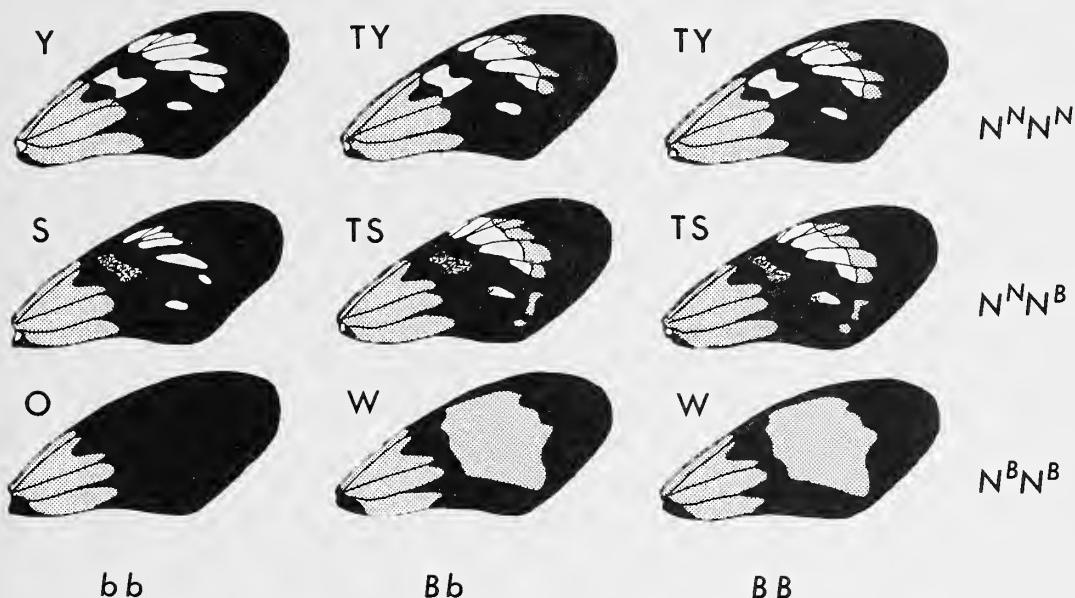
TEXT-FIGURE 4. The interaction of the B and N loci in determining the color of the band. About 0 times natural size.

S and Y, but the absence of O is not significant in a brood of only 6. A Y individual mated with a W butterfly known from its pedigree to be heterozygous Bb produced, as predicted, roughly equal numbers of S and TS (Table 7, brood 43). Brood 52 (Table 7) is a cross between S and TS (the bracket round the S in the table indicates that yellow marks were virtually absent); it segregates 4 TY, 12 TS, and 5 W, a satisfactory approximation to the ratio 1 : 2 : 1 expected if the TS parent was homozygous BB (from its pedigree it had an even chance of being homozygous).

The first generation backcrosses to Surinam (TS × Y) and to Trinidad (TS × W) also confirm the hypothesis. Those to Surinam (Table 2) gave, as expected, Y : TY : S : TS in the ratio 1 : 1 : 1 : 1 (actual numbers, including brood 46 from Table 7, are 33 : 46 : 43 : 29; $\chi^2_{(3)}$ = 5.2; P > 0.1). Those to Trinidad (Table 3) gave TS : W in the ratio 1 : 1 (actual numbers, including brood 16, are 64 : 70). The F_2 broods, produced by sib-mating F_1 hybrid butterflies (Table 4), segregate Y : TY : S : TS : O : W in the numbers (including brood 25) 4 : 27 : 9 : 38 : 0 : 17. The expected ratio from the hypothesis as shown in text-fig. 4 is 1 : 3 : 2 : 6 : 1 : 3, or in numbers 5.9 : 17.8 : 11.9 : 35.6 : 5.9 : 17.8. This gives χ^2 = 12.2 for 5 degrees of freedom, which is significant at the 5 percent level. The F_2 broods have therefore segregated all the expected phenotypes, except O, and the absence of this phenotype is mainly responsible for the significant deviation from the expected ratio. Reasons will be advanced later for thinking that the genotype bbN^BN^B may sometimes produce a phenotype very like TS; in that event the expected ratio is 1 : 3 : 2 : 7 : 3, which gives χ^2 = 6.4 for 4 degrees of freedom, which is not significant. The segregation of the F_2 broods therefore indicates that the hypothesis is probably correct, but that there are some additional complications which are not yet understood.

The genotype BBN^NN^N has not been formed for certain in these broods; reasons will be given later for thinking that it does indeed produce the phenotype TY.

Inheritance of shape of bands

The variation in the red bands (W or T) has been explained fully by the loci B and N. The yellow marks in the band may be either broken up into a series of spots (Plate-figs. 1-2, 5-7, 10-11, 14-16), or joined together into a yellow patch (Plate-figs. 3-4, 8-9, 12-13, 17-20). These phenotypes are indicated in the tables by superscript letters, F for a broken band and f (fused) for a joined one. This variation is found in the phenotypes Y, TY, S, TS, and probably O (the only two individuals obtained being apparently O^f). In the phenotype W (wide band), the variation is found in the distribution of white (or yellow) scales on the underside of the band, which are evenly spread in W^f phenotypes, but

TABLE 4. F₂ SURINAM × TRINIDAD HYBRIDS
(AND A SIMILAR BROOD)
(All Parents Have Phenotype TS^F Dr)

BROOD	MATING	PARENTS		DENNIS (Dr)										PLAIN (dr)										Total
				Y^F	Y^f	TY^F	TY^f	S^F	S^f	TS^F	TS^f	W^F	W^f	Y^F	Y^f	TY^F	TY^f	S^F	S^f	TS^F	TS^f	W^F	W^f	
22	M38	6	♂	1	1	1	1	1	0	3	0	1	0	0	0	0	0	0	0	1	1	0	0	21*
			♀	0	0	1	0	1	1	3	0	1	0	0	0	0	0	0	0	0	1	0	0	
23	M39	5	♂	0	0	4	0	1	1	3	1	1	0	0	0	0	0	0	0	0	0	0	0	6
			♀	0	0	0	1	1	1	3	0	1	0	0	0	0	0	0	0	0	0	0	0	
24	M40	5c	♂	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	2	0	0	0	22
			♀	0	0	2	0	0	2	3	1	1	2	0	0	1	2	0	0	0	0	0	0	
25	M42	1	♂	0	0	1	1	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	4
			♀	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
26	M47	5	♂	1	1	2	0	1	2	2	2	0	1	0	0	0	0	0	0	3	3	2	0	37
			♀	0	0	0	0	2	0	2	2	3	0	0	0	0	0	0	0	2	2	3	1	
27	M77	6	♂	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5
			♀	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	
			Total	2	2	14	4	5	4	18	6	7	6	0	0	3	3	0	0	8	5	3	1	94
			Total	4		18		9	9	24	13	13		9	9			0	0	14*		4		95*
			Total	64†																27*				91*†

* Includes 1 TSdr butterfly, not scored for F or sex.
† Excludes butterflies from brood 25.

TABLE 5. VARIOUS TEST-CROSSES

BROOD	MATING	PARENTS			RADIATE (DR)				PLAIN (dr)						Total
		♀	♂		TS^F	TS^f	W^F	W^f	TS^F	TS^f	TY^f	TY^F	W^F	W^f	
32	M59	1	Trinidad^b	♂ / ♀	0	0	0	0	0	1	1	0	0	0	2
33	M81	TY^F Dr Liverpool	W^f dr §34	♂ / ♀	0	0	0	0	0	0	1	0	0	0	1
34	M78	TS^F dr (combined progeny of 18 and 21)	T(–)dr	♂ / ♀	0	1	1	0	1	1	1	1	2	0	15*

* Includes the following, which could not be scored for F or f:
3 ♂ T(–)dr, 1 ♀ Wdr

TABLE 6. VARIOUS TEST-CROSSES

BROOD	MATING	PARENTS ♀	♂		DENNIS (Dr) TYF	TYf	TSF	TSf	WF	Of	PLAIN (dr) TYF	TYf	TSF	TSf	WF	Wf	Total
35	M55	41	41f	♂	0	0	0	0	0	0	0	0	0	0	0	1	3*
		WDr	Wdr	♀	0	0	0	0	0	1	0	0	0	0	0	0	
36	M91	49	49	♂	1	1	1	1	0	0	2	1	2	0	0	0	7
		TYFDr	TSfDr	♀	0	0	2	1	0	0	1	1	2	1	0	0	
37	M92	52	52	♂	0	0	0	0	1	0	0	0	0	0	0	1	7†
		WDr	WfDr	♀	0	0	0	0	3	1	0	0	0	0	0	0	
38	M95	52	52	♂	0	1	0	1	0	0	0	0	0	1	0	0	6
		TSfDr	TYfDr	♀	0	2	0	1	0	0	0	0	0	0	0	0	

* Includes 1 ♂ Wdr not scored for F or f.

† Includes 1 ♂ WDr not scored for F or f.

gathered into patches separated by red scales in W^F butterflies (Plate-figs. 24-25). The two types are often difficult to score on W butterflies, and in some butterflies they cannot be distinguished because there are so few white scales (columns headed W); TS phenotypes occasionally lack most of their yellow, but F or f can be detected on the underside as marks of a lighter brown than the background color.

Band shape is controlled by a single pair of alleles (F and f), with broken bands dominant to fused. Thus the F_1 hybrids from the cross $Y^F \times W^f$ (Table 1, broods 5-9) are always TS^F; the backcrosses to Surinam are entirely F (Table 2); and the backcrosses to Trinidad (Table 3) segregate 54F to 78f (or excluding the W phenotypes which are difficult to score, 29F to 33f). The F_2 broods, excluding brood 25, give 59F to 31f, or excluding the W phenotypes, 49F to 24f. The much closer correspondence to the expected ratio in the backcross when W phenotypes are excluded, shows that scoring on these butterflies is unreliable.

Inheritance of yellow line

"Yellow line" denotes a narrow band of scales starting at the base of the forewing and extending roughly along the posterior vein of the cell, towards the band (Plate-fig. 26). The subspecies *H. m. nanna* (with its variant population *H. m. burchelli*) is monomorphic for this phenotype (Plate-fig. 29), but in the present broods its expression is very variable, and it may be represented only by a yellow spot at the base of the wing; when it is combined with the dennis or radiate patterns, this dot is usually all that is visible. All the Surinam parents show the yellow line (in the form of the dot); it is absent in butterflies from Trinidad.

As one has to use a dissection microscope to score this character, only two of each of the backcrosses, one F_2 brood and a small sample from one F_1 have been scored. The butterflies are divided into three classes:

"++" The yellow at the base of the line is solid, with no mixture of black scales; the spot is visible to the unaided eye.

"+" The yellow scales are mixed with black scales, producing a vague yellow spot not immediately apparent to the unaided eye.

"—" The yellow line (or spot) is absent, or if present, the spot is represented by no more than half a dozen yellow scales.

The results (Table 8) show that the yellow line is a character of variable expression, particularly in TS phenotypes, but that it is strongly influenced by the N locus, such that $N^N N^N$ butterflies are usually "++", $N^B N^B$ butterflies "—", and heterozygotes variable but often "+". The other factors influencing this character have not been identified, but appear not to include F or sex, which for the sake of simplicity have not been tabulated.

Inheritance of white dots and yellow bar

The "white dots" are a series of faint white markings, comprising all or any of the following (Plate-fig. 27):

(a) a series of up to five white spots on the veins near the tip of the forewing about 3 mms from the edge of the wing;

(b) a white spot, rarely two, near the outer angle of the hindwing;

(c) a series of paired marginal spots at the posterior of the hindwing.

These marks are found only on the underside of the wings. They are not normally found in any of the races of *melpomene* in the Guianas, Venezuela, Trinidad, or Brazil, and I have never noticed them on any wild-caught specimen of any phenotype, although they are, of course, easily overlooked. All these marks are found in the closely related *H. ethilla,* and, all except the white spot on the hindwing, in various subspecies of the closely related *H. elevatus* (Turner, 1967).

Table 7. Various Test-Crosses

BROOD	MATING	♀	♂	Sex	RADIATE (DR) Y^F	Y^f	TY^F	S^F	S^f	TS^F	TS^f	W^F	DENNIS (Dr) Y^F	TY^F	TY^f	S^F	TS^F	TS^f	W^F	W	W^f	Total
39	M19	1 S^FDr	4 Y^FDr	♂	0	0	0	0	0	0	0	0	3	0	0	4	0	0	0	0	0	16
				♀	0	0	0	0	0	0	0	0	5	0	0	4	0	0	0	0	0	
40	M20	3 Y^FDr	1 Y^FDr	♂	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	21
				♀	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	
41	M24	2 S^FDr	Trinidad Y^FDr	♂	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	4	0	19
				♀	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	9	0	
42	M54	4 Y^FDr	3 W^fdr	♂	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	8
				♀	0	0	0	2	0	0	0	0	0	0	0	0	4	0	0	0	0	
43	M63	3 Y^FDR	Y^FDR	♂	2	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	9
				♀	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	
44	M64	3 Y^FDr	41^f WDr	♂	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	26
				♀	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	
45	M65	41 WDr	2^g S^FDr	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
				♀	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
46	M69	9 $T(S^F)DR$	1 Y^FDr	♂	2	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	11
				♀	2	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	
47	M71	2 S^FDr	2^g S^FDr	♂	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	6
				♀	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	
48	M76	3 Y^FDR*	3 Y^FDr*	♂	0	0	0	0	0	0	0	0	11	1	0	0	0	0	0	0	0	25
				♀	7	0	0	0	0	0	0	0	4	1	0	0	0	1	0	0	0	
49	M85	46 TY^FDr	34 $T(S)dr$	♂	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
				♀	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
50	M86	46 Y^FDR	46 Y^FDR	♂	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	6
				♀	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
51	M88	34 TS^FDR	?	♂	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4
				♀	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	
52	M90	46 S^FDR	34 $T(S)dr$	♂	0	0	0	0	0	2	1	0	0	1	0	0	0	2	1	0	0	21
				♀	0	0	2	0	0	2	1	0	0	0	0	0	1	3	0	0	0	
53	M96	52 TS^FDR	52 TS^FDR	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	7
				♀	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	0	
54	M43	Surinam^a Y^FDr	Trinidad W^fdr	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
				♀	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	

*The phenotypes of the parents may have been ♀ Y^FDr, ♂ Y^FDR.

TABLE 8. SEGREGATION OF YELLOW LINE IN A SAMPLE OF THE BROODS

BROOD	YELLOW LINE	RADIATE				DENNIS					PLAIN		
		Y	TY	S	TS	Y	TY	S	TS	W	TY	TS	W
5	++	–	–	–	–	–	–	–	9	–	–	–	–
	+	–	–	–	–	–	–	–	10	–	–	–	–
	–	–	–	–	–	–	–	–	2	–	–	–	–
11	++	0	6	0	0	3	11	3	0	–	–	–	–
	+	0	0	3	2	0	0	9	4	–	–	–	–
	–	0	0	0	0	0	0	0	0	–	–	–	–
13	++	6	7	0	0	6	8	0	0	–	–	–	–
	+	0	0	7	2	0	0	3	0	–	–	–	–
	–	0	0	1	3	0	0	3	5	–	–	–	–
20	++	–	–	–	–	–	–	–	0	0	–	0	0
	+	–	–	–	–	–	–	–	4	0	–	6	1
	–	–	–	–	–	–	–	–	0	8	–	0	8
17	++	–	–	–	–	–	–	–	0	0	–	0	0
	+	–	–	–	–	–	–	–	3	0	–	4	0
	–	–	–	–	–	–	–	–	10	20	–	11	13
26	++	–	–	–	–	2	3	0	1	0	4	0	0
	+	–	–	–	–	0	0	4	4	3	0	8	4
	–	–	–	–	–	0	0	0	0	1	0	1	0

The yellow bar appears to be the same as that found in *H. m. nanna,* extending across the hindwing in the region of the cell (Plate-fig. 29); in these broods it is represented only by a light sprinkling of yellow scales at the inner margin of the hindwing, and another sprinkling, in the form of a faint yellow stripe, beyond the outer end of the cell, or both these marks together (Plate-fig. 28). Usually it is stronger on the underside, but is sometimes visible on the upperside. It has been scored into three grades, "absent" (—), "weak" (+), and "strong" (++).

The white dots and yellow bar appear in a few butterflies in several broods, but only in brood 26 (Table 4), a cross between two TS^FDr butterflies both showing traces of the yellow bar, do these markings appear in a fraction of the brood sufficient for analysis. The segregation of brood 26 for these characters, and for band and dennis, is shown in Table 9: numerical results are extracted in Table 10; sex has no significant influence on the phenotype.

Tabulation of the white dots (absent versus present) against the segregation of the other characters (Table 10), shows that they are little influenced by the segregation of *F, D,* or *B.* But they are strongly influenced by the segregation of *N, $N^N N^N$* butterflies invariably showing the dots, the other two genotypes lacking

TABLE 9. SEGREGATION OF YELLOW BAR AND WHITE DOTS IN BROOD 26

YELLOW BAR	NONE	WEAK			STRONG		
WHITE DOTS	No White Dots	No White Dots	Hindwing Spot + Marginals	All White Dots	Forewing Apicals	Hindwing Spot + Forewing Apicals	All White Dots
PHENOTYPE	F f	F f	F f	F f	F f	F f	F f
YD	0 0	0 0	0 0	1 1	0 0	0 0	0 0
TYD	0 0	0 0	1 0	0 0	0 0	1 0	1 0
SD	1 2	1 2	0 0	0 0	0 0	0 0	0 0
TSD	0 0	4 0	0 0	0 0	0 0	0 0	0 0
WD	0 0	3 1	0 0	0 0	0 0	0 0	0 0
TYd	0 0	0 0	0 0	0 0	1 0	3 0	0 0
TSd	3 4	2 0	0 0	0 0	0 0	0 0	0 0
Wd	0 0	2 2	0 0	0 0	0 0	0 0	0 0

them. There is also a strong association between the white dots and the yellow bar.

The association of yellow bar with the segregation of the N locus is less clear-cut than that of the white dots, but Table 10 shows that it is considerably influenced by the N locus and the D locus, the alleles N^N and D tending to increase the size of the yellow bar.

The white dots and yellow bar, like the yellow line, are thus in some broods influenced by segregation at the N locus, and the yellow bar in addition by the D locus; but all these characters are very variable in expression and influenced by other factors. The high frequency of the yellow bar and white dots in one brood only, suggests that at least some of these other factors are genetic.

LINKAGE

None of the loci B, D, F, N is sex-linked, as is plainly shown by segregation in all the large broods. Autosomal linkage can be investigated as follows:

B and N: The even segregation in the backcrosses to Surinam (Table 2) and in brood 1 (Table 1) shows that these loci are unlinked, as does the F_2 (Table 4) whose segregation for these loci was discussed above for its fit to a $1 : 3 : 2 : 6 : 1 : 3$ ratio. These figures, tested as a 2×3 contingency table give $\chi^2_{(2)} = 3.9$, which is not significant.

N and D: The backcrosses to Trinidad segregate

	Male parent heterozygous			Female parent heterozygous	
	Dr	dr		Dr	dr
TS	18	21	TS	10	17
W	25	18	W	12	13

(DR included with Dr); the F_2 segregates (excluding brood 25)

	Dr	dr
Y +TY	20	9
S + TS	31	14
W	13	4

None of these segregations shows any sign of linkage.

N and F: The backcrosses to Trinidad segregate

	Male parent heterozygous			Female parent heterozygous	
	TS	W		TS	W
F	19	15	F	13	9
f	21	28	f	12	17

and the F_2:

	Y + TY	S + TS	W
F	20	29	10
f	9	15	7

Again there is no sign of linkage.

F and B: As f does not segregate in the Surinam backcross, nor b in that to Trinidad, only the F_2 can detect linkage:

	F	f		F	f
Y+S	7	6	Y+S	7	6
TY+TS+W	52	25	TY+TS	42	18

The test is not very efficient because the genes would be in repulsion, if linked, but the loci appear to be unlinked, for there is a slight excess of "recombinants" over expectation.

D and B: Again, only the F_2 tests these loci, and it is not possible to exclude the possibility, described above, that some bbN^BN^B individuals appear the same as BbN^NN^B or BBN^NN^B. The segregation is

TABLE 10. ASSOCIATION OF YELLOW BAR (SCORED AS ABSENT, WEAK, AND STRONG) AND WHITE DOTS (SCORED AS ABSENT OR PRESENT) WITH OTHER CHARACTERS IN BROOD 26

YELLOW BAR	GENOTYPE								
	$D-$	dd	N^NN^N	N^NN^B	N^BN^B	$B-$	bb	$F-$	ff
–	3	7	0	10	0	3	7	4	6
+	14	6	3	9	8	5	15	14	6
+ +	2	4	6	0	0	0	6	6	0

WHITE DOTS	YELLOW BAR			GENOTYPE								
	–	+	+ +	$D-$	dd	N^NN^N	N^NN^B	N^BN^B	$B-$	bb	$F-$	ff
–	10	17	0	14	13	0	19	8	6	21	16	11
+	0	3	6	5	4	9	0	0	2	7	8	1

	Y + S	TY + TS + W
Dr	13	51
dr	0	27

giving $\chi^2_{(1)} = 6.4$, which is significant at the 2 percent level (one tailed). It therefore seems very likely that D and B are linked, because no recombinants appear in the F_2. As this is a repulsion F_2, apparently with close linkage, there is little point in estimating recombination.

Brood 46 (Table 7) was set up to test this, an individual of genotype BbD^Rd from the F_1 being backcrossed to a Y^FDr Surinamian butterfly. In this cross, as distinct from the other Surinam backcrosses using $BbDd$ individuals, both the D and B loci can be seen segregating. Unfortunately the brood is small, producing 6 bbD^RD parental types, 5 $BbDd$ parental types and no recombinants. The probability of getting this result by chance if the genes were unlinked is 2.5×10^{-8} (Fisher's exact test, one tailed), and the 95 percent confidence limit for the combination fraction is 15.5 percent (on the assumption that if one more butterfly had emerged it would have been a recombinant). The loci B and D are therefore linked, and the recombination fraction between them may be low, especially as the recombinant phenotypes Sdr and Odr are not known in the wild. It will be shown later that recombinations probably do occur.

D and F: This is the only relationship which presents difficulties. The F_2 gives no indication of linkage (brood 25 excluded):

Including W			Excluding W		
	D	d		D	d
F	42	17	F	35	14
f	22	9	f	16	8

Both values of χ^2 (0.0005 and 0.17) are small and not significant. The backcross to Trinidad likewise gives no sign of linkage when the heterozygous parent is male (Table 3, broods 16-18):

Including W			Excluding W		
	D	d		D	d
F	16	16	F	9	8
f	27	22	f	9	12

On the other hand, when the heterozygous parent is female, we have

Including W			Excluding W		
	D	d		D	d
F	15	7	F	10	3
f	7	22	f	2	10

Here, $\chi^2_{(1)}$ is respectively 9.89 and 9.08. In addition, brood 52 (Table 7) segregates D^R and D, F, and f, and although the male parent cannot be scored for F, it is clear that the female is doubly heterozygous D^RDFf. The brood gives

	DR	Dr
F	8	3
f	2	8

for which $\chi^2_{(1)} = 5.84$. All χ^2 values are one-tailed and significant, and both segregations give a recombination fraction of 26% $\pm$ 16% (95 percent confidence limit includes 50 percent recombination). It is therefore possible, but not completely proven, that the loci recombine freely in males but are linked in females. The F_2 segregation provides a further check. Using formulae given by Bailey (1961) we can calculate

$$\theta = (1 - r_1)(1 - r_2)$$

where r_1 and r_2 are the recombination fractions in males and females. Taking r_1 as 50 percent, we can estimate r_2 if we know θ:

$$r_2 = 1 - 2\theta$$

Calculating from the data, using Bailey's quadratic formula, $\theta = 26\% \pm 7\%$, and hence $r_2 = 48\%$, with a lower 95 per cent confidence limit of 21 per cent. The F_2 therefore indicates that the recombination fraction in females is 50 percent (no linkage), but leaves open the possibility that it is as low as the 26 percent estimated from the backcross.

The broods therefore lead us to conclude that D and F are unlinked, but leave open the possibility of moderate linkage or of disturbed segregation in females. It will be remembered that B and D are linked, and that F shows no linkage with B, which argues that D and F are indeed not linked.

Effect of Genetic Background

The band

One male butterfly from brood 5 sired three broods, an F_2 and both types of backcross; the TS phenotypes in these broods therefore enable us to investigate the effect of the gene-complexes of the Surinam and Trinidad subspecies on the expression of the genes affecting the band.

The parental male, a random sample of his sibs, and all TS butterflies from the F_2 and both backcrosses are shown in plate-figs. 30-33. It is clear from this that the loci D and F (or genes on the same chromosomes) affect the amount of red and yellow in the band, d enhancing red and reducing yellow, and f enhancing red (its effect on yellow, being its major characteristic, has already been described). Within the TS^{F}Dr phenotypes, butterflies from the Surinam backcross show slightly more yellow and less red than those in the Trinidad backcross, but the difference is very slight and no doubt would not be significant if tested statistically.

There is thus little doubt that the Trinidadian alleles at the D and F loci (or loci on the same

chromosomes) change TS phenotypes in the direction of the Trinidadian subspecies (less yellow and more red on the upperside), and that the Surinamian alleles have the reverse effect, changing them towards the Surinamian subspecies; other loci may have a similar effect, but in these broods at least it is weaker.

Radiate

The radiate phenotype also seems to be influenced by other loci, as a few butterflies have the hindwing rays expanded into wide wedges which almost touch at their sides (Plate-fig. 34); the rays also appear as prominent red streaks on the underside (normally they are thin and barely noticeable). Many butterflies in the broods in which this variation appeared are now lost, for reasons explained above, so a full analysis is not possible. Of the surviving butterflies, the following show the "spread ray" phenotype:

Broods 21 & 34: 2 out of 6 radiate
Brood 50: 1 out of 5 radiate
Brood 52: 1 out of 2 radiate

As this phenotype does not occur in the pure Belém subspecies (*H. m. thelxiope*) (Text-fig. 1a), nor in the F_1 or Surinam backcrosses, but has appeared in broods 21 and 34, the only Trinidad backcrosses containing radiate, it seems very likely that it results from a gene or genes present in the Trinidadian but not Surinamian stock, and more or less recessive in their effect.

Sex

Plate-figs. 35-36 show the effect of sex on the S^F phenotype in brood 1; in this brood the sexes clearly differ in the amount of yellow. The effect has not been noticed in other broods.

DOUBLE MATING

A Surinam female·layed 158 eggs between September 28 and November 6; all of those surviving (Table 1, brood 3) were the offspring of her wild radiate mate. Mated accidentally to a Trinidadian male on November 7, she laid one egg on November 8 and then died; this offspring (Table 7, brood 54) is obviously the offspring of her wild mate and not of the Trinidadian male.

DISCUSSION

Identity of the genes

Loci designated by *B, D,* and *N* are described by Turner and Crane (1962) and Sheppard (1963) in breeding experiments with *H. melpomene* from Surinam and Trinidad. It is necessary to show that the loci described here are indeed the same loci, in so far as this can be done without hybridizing the strains used in the various experiments. The *F* locus, althoug it seg-

regated in the broods of the previous authors, was not described by them.

There can be little doubt that the alleles D^R, D and d, and N^N and N^B are the same as those described by Sheppard because phenotypes, dominance, and linkage relations are the same. The only points of difference are that Sheppard did not demonstrate the allelism of D^R and D, and that his genotype $N^N N^N$ was homozygous *BB*; this genotype has not been formed for certain in the present experiments, and its phenotype has been inserted in text-figure 4 (top right) on the strength of Sheppard's results. Turner and Crane describe also the D locus (which they attribute to a series of chromosomes DR, Dr, and dr); there can be no doubt that this is the same locus. *N* did not segregate in their broods.

The only locus about which there is any doubt is *B*. Turner and Crane describe a recessive allele *b*, linked to *D* (recombination fraction unknown, although crossovers occurred), which reduces the amount of red in the forewing band. The *b* allele in the present paper is linked in the same way and reduces the amount of red in the band. The difference is that in the experiments of Turner and Crane, the genotype $bb N^B N^B$ had a narrow red band edged with dusky yellow (the TS phenotype), while in the present broods this genotype virtually lacks the band altogether (the O phenotype) (Plate-fig. 20). There are three possible explanations of this:

(a) there are two separate loci, both linked to *D*, which produce this effect;

(b) there are two recessive alleles at the *B* locus, both reducing the red but to a different extent;

(c) the same allele appeared in both experiments, but its effects were altered by genes at other loci.

There is a small amount of evidence in favor of this last hypothesis, in that the amount of red of TS phenotypes is altered by the *D* and *F* loci, and possibly by others (see above). S phenotypes occasionally have small amounts of red in the band, suggesting that other genes can alter the amount of red present. Further, in brood 50, a cross between two Y individuals, a TY phenotype appeared. This may have been an error, or it may indicate the segregation of factors enhancing the red. The *B* locus did not segregate in Sheppard's broods.

Variable expression

In the above broods, the $N^N N^N$ genotype does not always produce the extra yellow and white marks (yellow line, yellow bar, and white spots). To understand this we must remember that none of the genes described in this paper is solely

responsible for the presence or absence of any particular pattern; it only co-operates with a number of other loci in producing its effects. Clearly several segregating loci affect the yellow bar and yellow line, and only when the genetic make-up of the individual is correct at these loci do we find the N locus producing and removing the marks. In most broods the genotype at the other loci is such that the markings cannot be produced by the $N^N N^N$ genotype.

The yellow bar sometimes appears in wild *melpomene* in the Guianas; one of these rare individuals is illustrated in Plate-fig. 37.

The same considerations apply to the influence of sex on the S^F phenotype in brood 1.

Recombination

Suomalainen (1965) has shown that chiasmata are absent at meiosis in female moths; if this is so in butterflies, then brood 46 could not have shown recombination, as linkage between loci on the same chromosome would be absolute. The presence of chiasmata in *melpomene* is now being investigated; future breeding experiments must always attempt to measure recombination in both males and females. If there is no recombination in females, it follows that the apparent recombination fraction of 25 percent between D and F must be spurious.

Conclusions

The polymorphism of *H. melpomene* in the Guianas is controlled by at least four loci, B, D, F, and N, affecting the distribution of red and yellow marks on the wings, with a further possible locus affecting the amount of red in the band. In addition, substitutions at other loci affect the expression of the four major loci, broadening the rays produced by the allele D^R, causing the allele N^N to produce extra yellow and white marks, and altering the amount of yellow in the band. The loci B and D are the only ones which are linked, although D itself may consist of two linked loci.

Among the homozygous genotypes are bbD^R-$D^RFFN^N N^N$, $bbDDFFN^N N^N$, and $BBddffN^B N^B$, which give the three phenotypes in text-figure 1. It is shown elsewhere (Turner, 1971b) that these are monomorphic subspecies, and that their hybridization accounts for the polymorphism of this species in the Guianas.

Summary

1. *Heliconius melpomene* from Trinidad, West Indies, were crossed with *melpomene* from a slightly polymorphic population belonging to another subspecies from central Surinam.

2. The presence or absence of red marks at the base of the wings is controlled by three alleles at a single locus (D).

3. The amount of red and yellow in the band on the forewings is controlled by the interaction of two loci (N and B).

4. The distribution of yellow pigment in the band is controlled by a single locus (F).

5. B and D are linked at not more than 16 percent recombination, and the other loci are unlinked, although F and D show some irregular segregation.

6. Three characters not found in the parent stocks appeared in some of the hybrids; these

were controlled chiefly by the *N* locus. The characters are normally found in various close relatives of *H. melpomene*.

7. There may be an additional locus enhancing the amount of red on the forewing.

8. The amount of red and yellow in the band is influenced by the *D* and *F* loci, and further loci cause the ray marks on the hindwings to fuse together when they are backcrossed into Trinidadian stock.

9. The three Guianian subspecies of *melpomene* are probably homozygous in different ways for alleles at the four major loci, the Surinam and Trinidad races differing by one substitution at each locus.

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EXPLANATION OF THE PLATES

1   2

3   4

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 1-2 Y^F
Figs. 3-4 Y^f

5   6

7

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

    Figs. 5-7   TY^F

8

9

10

11

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 8-9   TY^f
Figs. 10-11   S^F

12

13

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 12-13 S^f

14

15

16

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 14-16   TS^F

17

18

19

20

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 17-19   TS[f]
Fig.   20   O[f]
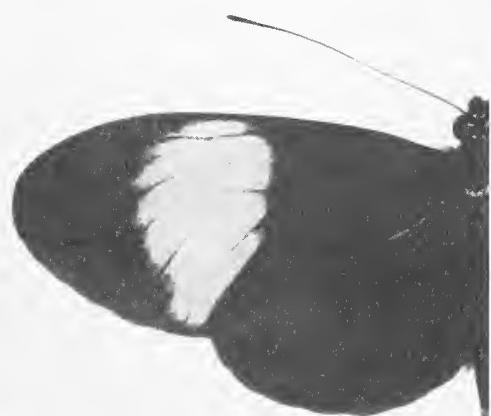
FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 21-23   W

FIGURES 24-25. The effect of the F locus on the distribution of white scales on the underside of the W phenotype.

    Fig. 24 W^F
    Fig. 25 W^f

FIGURE 26. The yellow line pattern
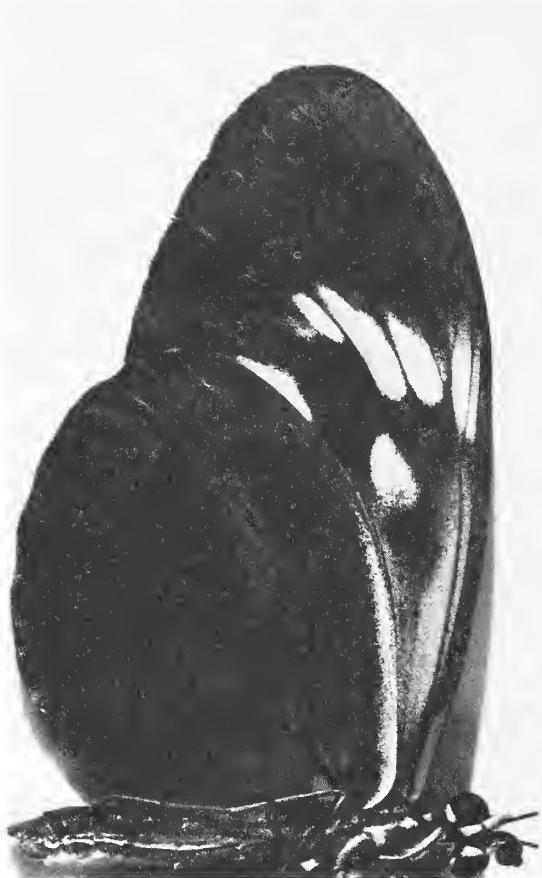
FIGURE 27. The white dots

FIGURE 28. The yellow bar
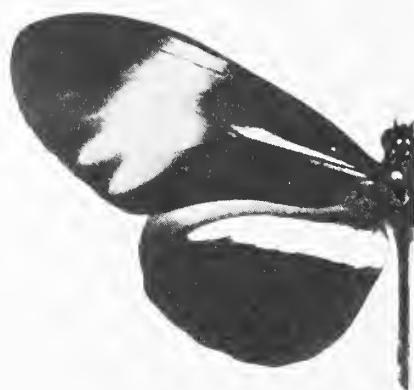
FIGURE 29. The Brazilian subspecies *H. melpomene nanna*
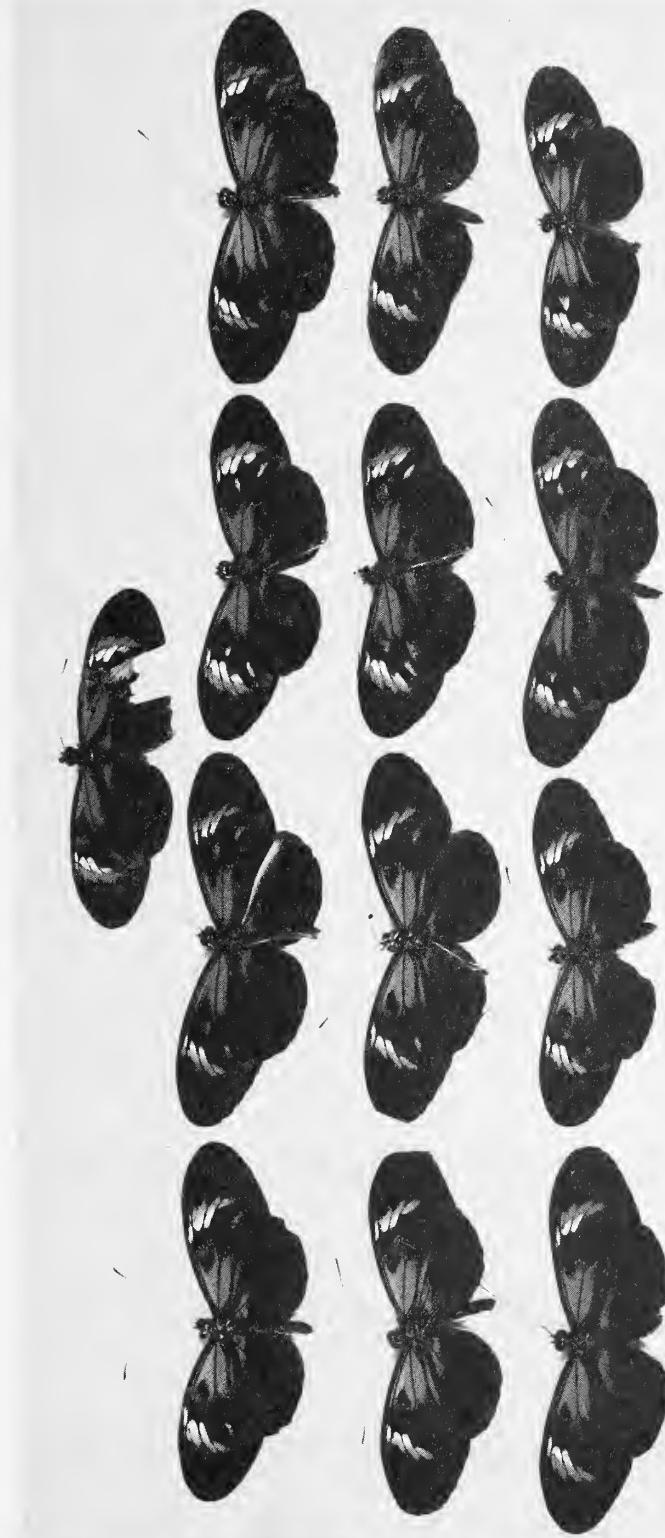
FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male

Fig. 30 The male F_1 parent and a few of his sibs (brood 5)
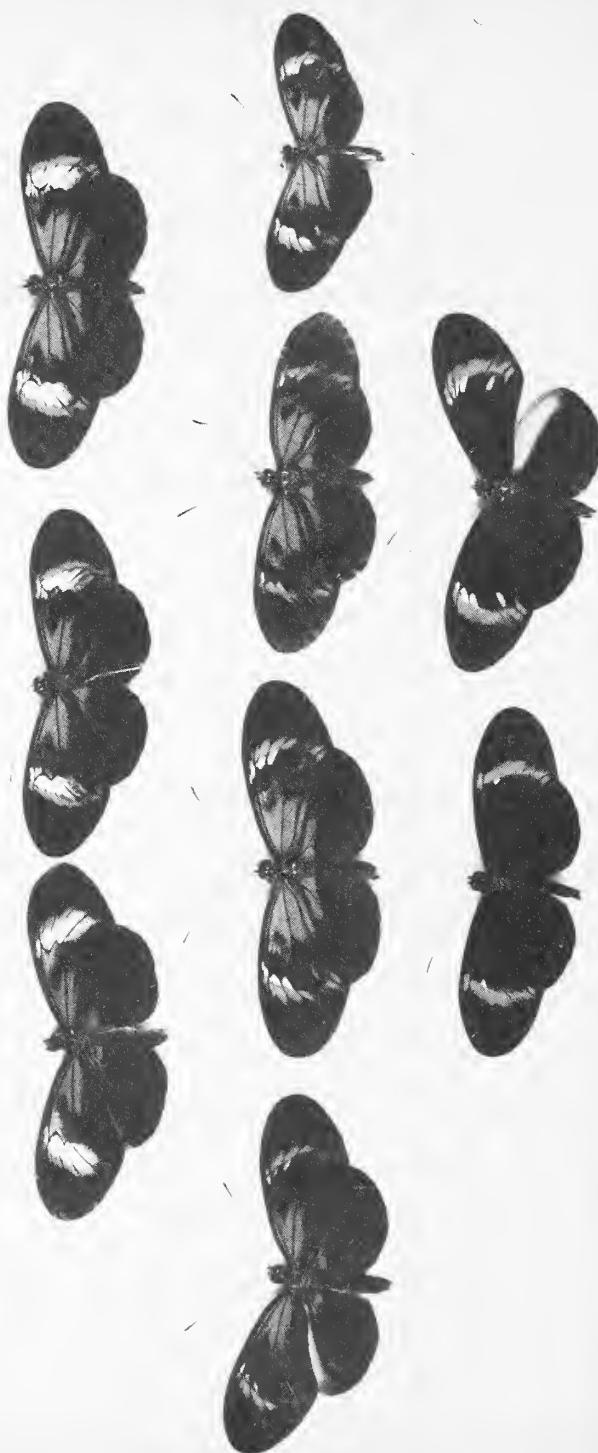
FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male
Fig. 31   The F$_2$ (brood 24)
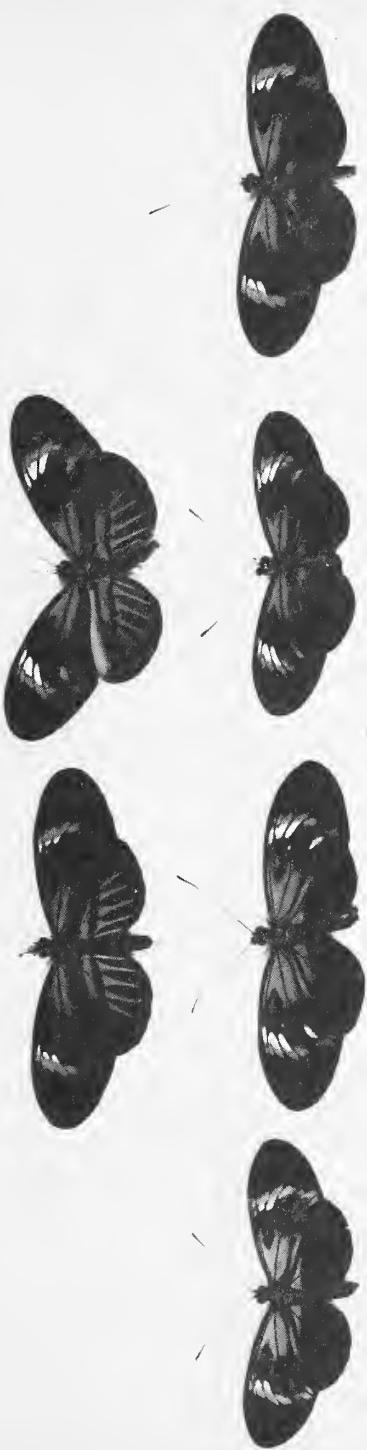
FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male
Fig. 32   The backcross to Surinam (brood 11)
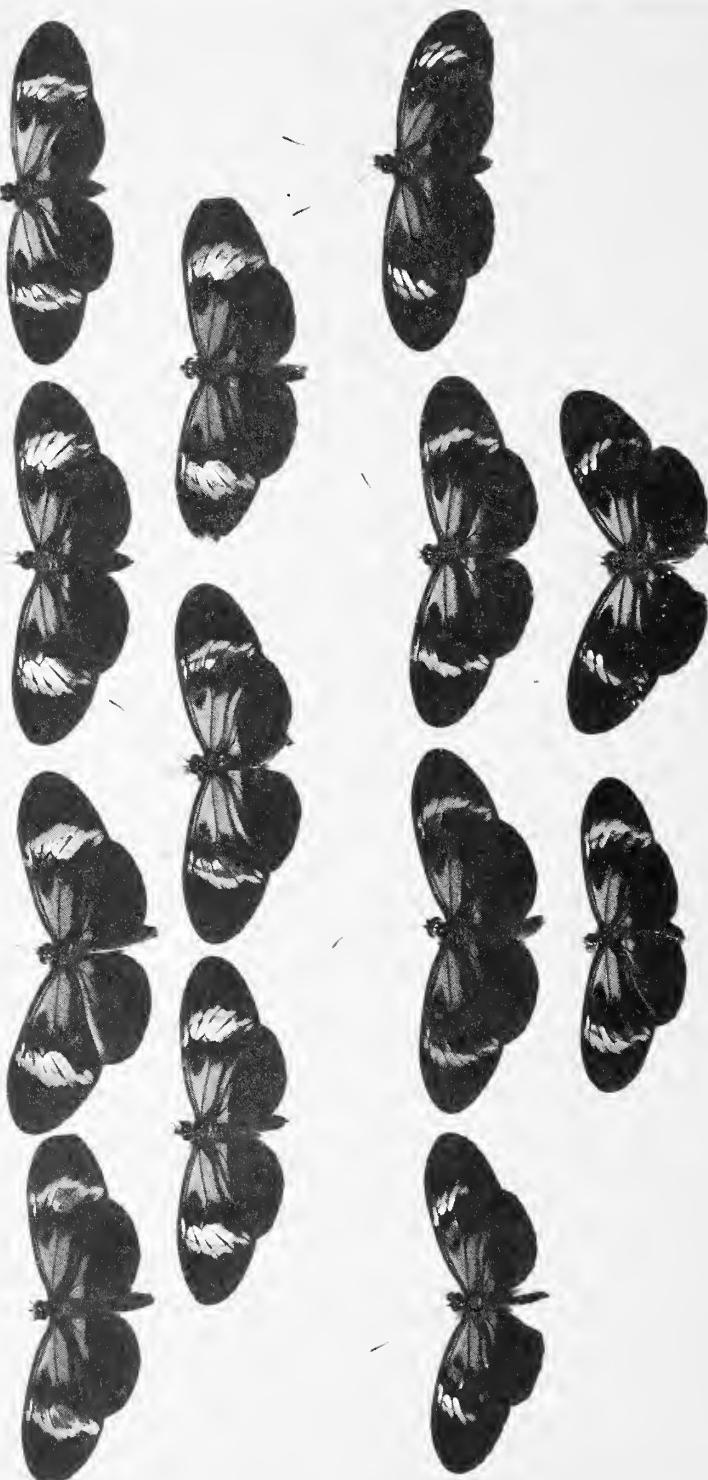
FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male
Fig. 33   The backcross to Trinidad (brood 17)

Figure 34. A phenotype with the ray marks partly fused together

Figure 35. Male S^F phenotypes in brood 1
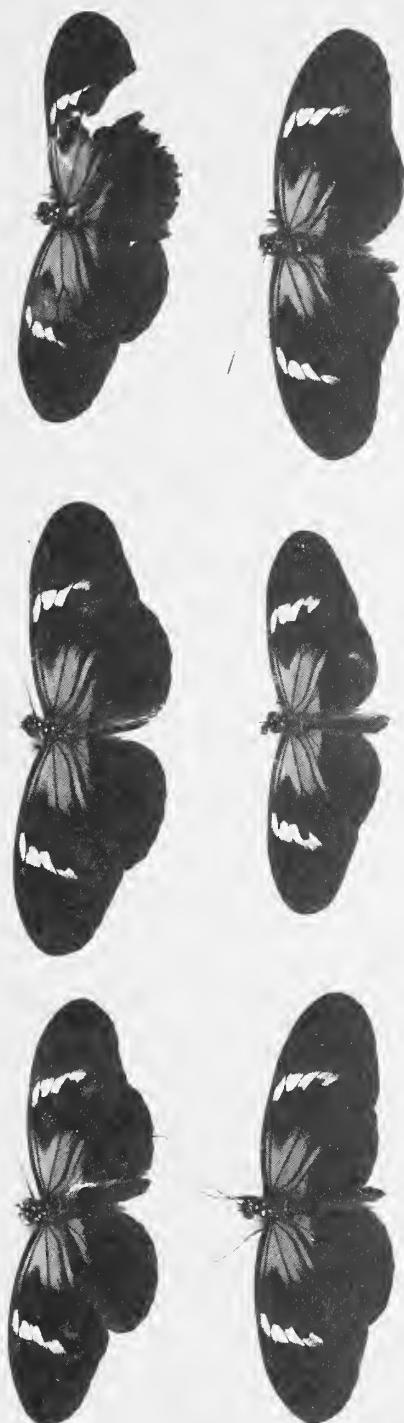
FIGURE 36. Female S^F phenotypes in brood 1

FIGURE 37. The type figure of var. *elringhami* J & K (by permission of the Royal Entomological Society of London) (from Joicey and Kaye, 1917).
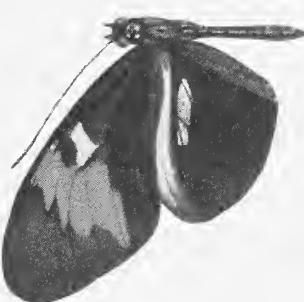
NEWS AND NOTES

Underwater Sounds of Southern Right Whales

(Plate I; Text-figures 1-2)

Long, repeated vocalizations are made by two species of baleen whales, *Megaptera novaeangliae* (Payne and McVay, 1971) and fin whales, *Balaenoptera physalis* (Walker, 1963; Walker, 1964; Patterson and Hamilton, 1964; Schevill, Watkins, and Backus, 1964; Weston and Black, 1965; Kibblewhite, Denham, and Barnes, 1967; Northrup, Cummings, and Thompson, 1968). A preliminary study of the vocalizations of a third baleen whale species, the southern right whale *Eubalaena glacialis,* is reported here.

On the basis of a report from the R/V Hero (Gilmore, 1969), the New York Zoological Society sponsored a preliminary study of a small concentration of southern right whales near Peninsula Valdez, off the coast of Argentina in September 1970. The purpose of this study was to record their vocalizations and to lay the groundwork for a more prolonged study of their behavior in subsequent years, should their location prove amenable to such studies. This paper will be concerned with their sounds.

During approximately 12 hours of recording from a 4-meter boat within one kilometer of a group of whales, a variety of sounds in the range audible to humans were recorded. Some of them are extremely similar to sounds recorded from right whales by Schevill and coworkers (Schevill and Watkins, 1962 (record); Schevill, 1962 (*Oceanus*); Schevill, Backus, and Hersey, 1962). In fact, the first and second sounds in Group B which we present here are almost identical to a spectrogram given by Schevill (1962), and again by Schevill and Watkins (1962).

On the other hand, the sounds that we recorded differ considerably from the sequential sounds in "stanzas" presented as right whale sounds by Cummings and Philippi (1970) (their basis for species identifications is not indicated). Some of their spectrograms are very similar to spectrograms of our recordings of the lowest humpback whale vocalizations. Since their recorder was limited to frequencies below 175 Hz, many frequency components of the true sonic emissions may be missing in their spectrograms. The periods of silence between bouts of low

sounds in their records correspond appropriately to bouts of higher frequency sounds in humpback songs—signals which would have been too high to have been recorded by Cummings and Philippi. It seems possible, therefore, that these sounds could have been made by humpback whales.

Our sample of right whale sounds comes from about nine hours recorded in early afternoon on three different days, and three hours recorded one night. We recorded the kinds of sound described here both in open ocean and in an enclosed bay. In all cases our hydrophones were within one kilometer of a group of right whales. In the daytime the sounds were infrequent, about one isolated sound per half hour. The sample recorded at night shows at least one sound every minute, and occasionally clusters of up to 15 sounds per minute. We do not know whether the increased vocal activity at night was accompanied by increased physical activity.

Pronounced differences in intensity of adjacent sounds in some of our recordings suggest to us that several whales were vocalizing at different distances from the hydrophone. Some sounds occurred in groups, the components being a few seconds apart and roughly equal in intensity, with a typical group lasting no more than one minute, and containing up to 15 sounds. It seems likely that all the sounds in such a group were made by one whale. Single utterances surrounded by long silences were also common.

In no case did we hear a long, continuous, patterned sequence of sounds, and we concluded that the whales were not singing songs at this time (late September) and place. However, this does not necessarily imply that these whales do not sing at other times of year. For example, although the songs of humpback whales can be heard almost constantly in the waters near Bermuda during April and May, the humpbacks that feed in New England waters have not been heard singing songs during the summer and fall (Schevill, 1964). During that period, only less highly organized, more isolated vocalizations have been recorded. The same sort of pattern could exist in right whales, and the fact that the nonrepetitive organization of right whale sounds we report here does not parallel humpback

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songs or the stanzas which Cummings and Philippi describe for right whales does not mean that such stanzas or songs are not sung by right whales.

The samples shown here were recorded between 1 a.m. and 4 a.m. on September 23, 1970, with a hydrophone which hung over the side of a small boat. The hydrophone was amplified and played in to one channel of a Sony 770-2 tape recorder. The recording apparatus was uniform in response within ±2 dB from 20 Hz to 15 kHz. Spectrograms were made on a Kay model 7029A spectrograph.

The recordings contain much wave- and boat-generated noise, seen as vertical bands on the spectrograms. Text-fig. 2 is a tracing of the spectrograms in Text-fig. 1, made to guide the reader to the whale-generated sounds among the noise. Since many structural details in the spectrograms are lost in noise, especially when the sounds are pulsive, these tracings are quite subjective. They do not substitute for spectrograms and are only intended to indicate the general nature of each sound, to draw attention to the fundamental frequency of tonal sounds, and (by omission) to identify artifacts. We have left out all harmonics except in the case of sounds which are pulsive or rasping and which therefore must depend heavily on their harmonic structure for their acoustic effect on the human ear. We hope thereby to make clear the major features of our categories of different types of sounds.

Group A and Group B show the units in two groups of sounds. The numbers between the spectrograms indicate intervals of silence (in seconds) between the end of one sound and the beginning of the next. Five sounds were omitted from Group A because they were much weaker and contained fewer high harmonics than similar sounds in other groups, and thus were judged to come from a more distant whale. (The missing sounds occurred between the first and second spectrograms, the second and third, the eighth and ninth, and the ninth and tenth [two sounds]). No sounds were omitted from Group B.

Examples C, D, and F show isolated sounds which (as far as we can hear from the recording) were surrounded by three to 30 minutes of silence. The most common type of isolated sound in our sample is exemplified by D and E. E′ is a spectrogram of the sound in E, shown on an expanded scale.

The briefest overall examination of the sample spectrograms shows that the vocabulary of these whales is complex, even in such a small sample (in fact our sound types may prove to be points along a continuum). The units in a group of sounds differ from each other in form and in the amount of time that separates them. The

organization of units within a group does not seem to be stereotyped.

In frequency, most of the sounds seem to lie between 50 Hz and 500 Hz, but occasional clear sounds as high as 1500 Hz were heard: two examples are shown in spectrograms C and I. Schevill, Backus, and Hersey (1962) reported no fundamental frequencies over about 400 Hz in *Eubalaena glacialis* near Cape Cod and, as mentioned, the hydrophone used by Cummings and Philippi was insensitive above 175 Hz. While it is possible that some other source was responsible for the 1500 Hz sounds we recorded, they were present in the same times, places, and rough relative intensities (subjective interpretation) as the lower sounds, and they sometimes occurred in groups whose other components were low sounds. The evidence suggests that both the high and low sounds were made by right whales.

The frequency span of each sound is narrow (usually less than one octave) but the differing harmonic structure adds much variety to the sounds. There are constant fluctuations between pulsive sounds (i.e., sounds rich in harmonics, giving a rasping, atonal quality), and simpler sounds containing a recognizable, clear pitch.

The bottom line of Text-figure 1 and Text-figure 2 gives samples of different types of sounds, with units II, III, IV, and VI presented on an expanded time scale to show the pulsive structure better. Some sounds seem to be entirely nonpulsive (e.g., I; also C, and the first unit in Group B). Some start as tonal and end rasping (e.g., II; also the third unit in Group A). Some start pulsive or rasping and end more tonal (e.g., III; also the fifth unit in Group B). Others start and end pulsive with a tonal section in the middle (e.g., IV). Still others have a clear pitch at the beginning and ending and a pulsive section in the middle (e.g., V). Some are pulsive throughout (e.g., VI). Some seem to have more than one fundamental, and a correspondingly complex harmonic structure (e.g., VII), but better signal-to-noise ratios are needed to confirm this. As both Group A and Group B indicate, many types of structure may be included in a group of sounds.

The function of these sounds is not known. The whales kept in a fairly close group including both mature and very young animals, and much social activity was going on. In one area there were cliffs from which we were able to observe clearly through the water the behavior of whales swimming directly beneath us. We witnessed on three occasions what appeared to be postural behavior that occurred on meeting. Having once seen it at close range, we could see that it often occurred when two whales met. In all three instances in which we saw it clearly, a

whale lying motionless at the surface of the water with its back exposed was approached slowly (at less than 1 km/hour) from the rear by a deeper whale. As the approaching whale passed beneath, the stationary whale flexed its spine so it curved laterally while it was also thrown into a sinusoidal curve in the vertical plane—a very strange and awkward movement visible from far away. Such a greeting is shown in the three photographs of Plate 1. In addition to this greeting ceremony, we also watched what we believe to be feeding, nursing, mating* and frequently playing—sometimes between mother and yearling calf (age judged by size), but more often by a calf alone. We never observed play between two calves, even during what seemed like a likely occasion when two adults, each with young, lingered next to each other for about two hours.

SUMMARY

Underwater recordings made near several groups of right whales show a variety of sounds, principally in the range 50 Hz to 500 Hz but including some as high as 1500 Hz, not previously reported for this species. The frequency range of each sound is narrow, but the harmonic structure of some is complex. Both single sounds and sounds occurring in groups lasting up to one minute were recorded. The irregular and nonrepetitive organization of sounds in groups indicates that these are not "songs," and different intensities in adjacent sounds suggests that more than one whale was involved. Vocal activity was greater at night than in daytime. Some behavioral observations were made.

ACKNOWLEDGMENTS

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* A paper describing these activities is in preparation as part of a planned intensive program over the next few years to extend these observations in the same area, while making simultaneous sound recordings, and to attempt to link some of the sounds to behavior.

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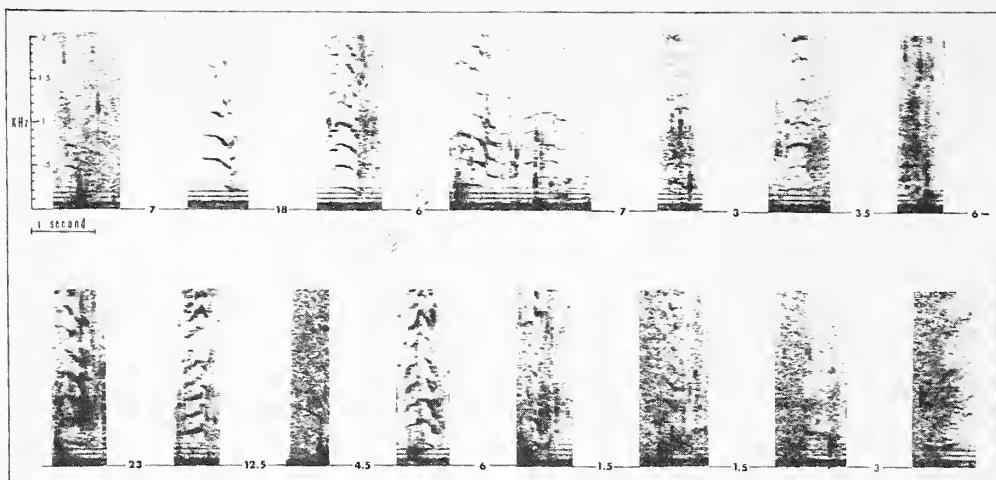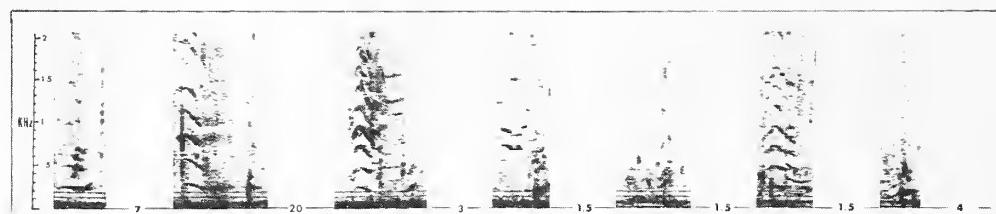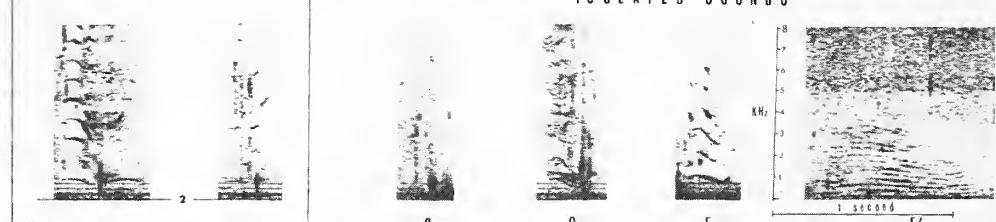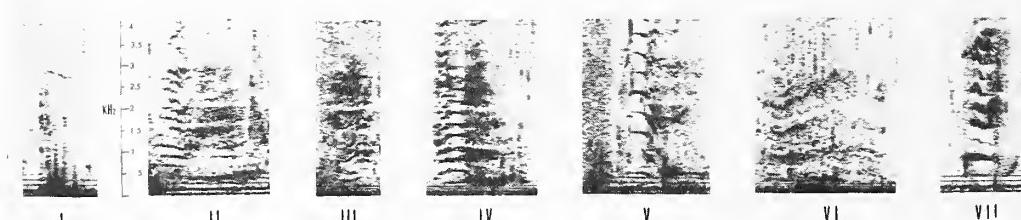
TEXT-FIGURE 1. Spectrograms of sounds recorded from right whales. Group A and Group B are two series of sounds presented in the order of their occurrence; the numbers between spectrograms indicate the duration in seconds of the silences between sounds. C, D, and E are characteristic single utterances surrounded by long silences. E and E′ are spectrograms of the same sound. The effective filter bandwidths are as follows: in E′ 45 Hz; in II, III, IV, and VI 22 Hz; and in all others 11 Hz. The bottom row (I-VII) is a selection of sounds to represent the variety of sounds made by right whales, and are drawn from different points in our records (see text). The scale preceding spectrogram II applies to examples II, III, IV, and VI.
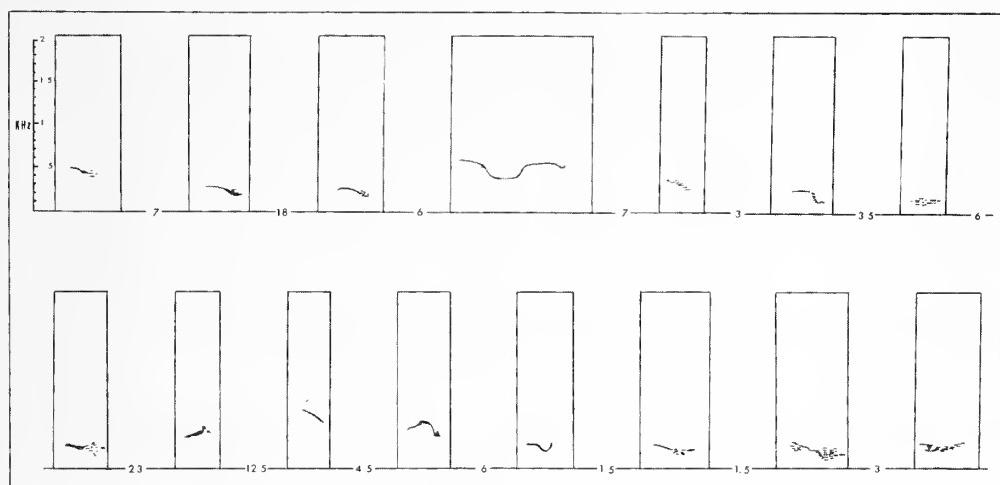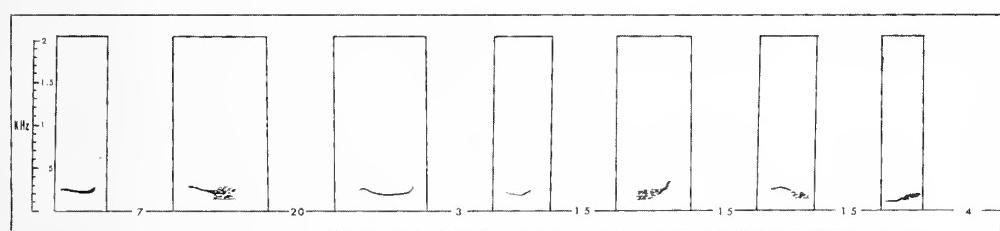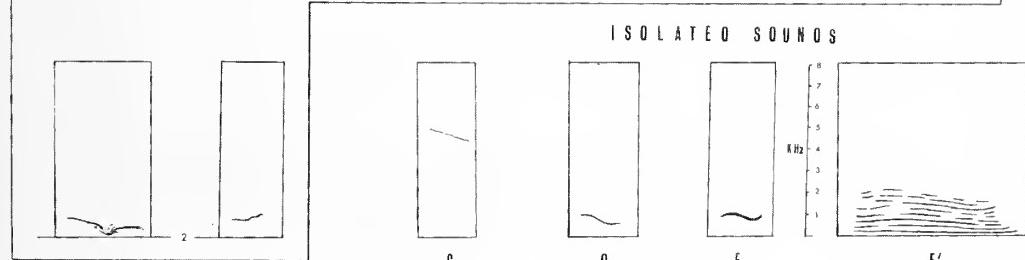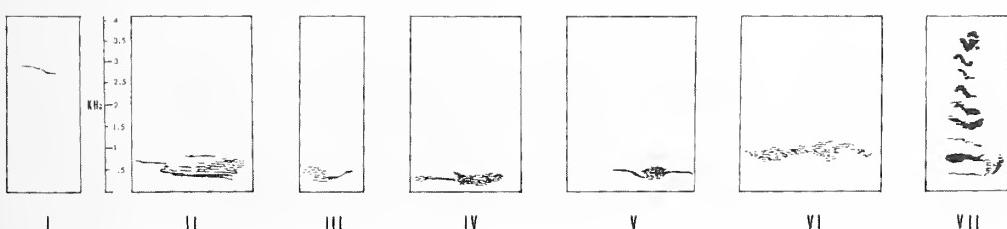
TEXT-FIGURE 2. A diagrammatic tracing of the spectrograms in Text-figure 1, omitting all but some of the components of the whale sounds (see text). The purpose here is to try to indicate the quality of the sounds when heard by the human ear. This is done by omitting harmonics and drawing just the principal frequency for moans and other somewhat tonal sounds. For atonal, pulsive, or rasping sounds, their many characteristic harmonics are included. The different types of sounds produced by right whales are more apparent from this presentation, but it is not intended to imply what features of these sound, if any, are attended to by the whales.

EXPLANATION OF THE PLATE

PLATE 1

Postural behavior often seen between two whales. This time it concerned a female accompanied by a calf being approached by a third whale. See text for explanation. Note that the calf is closer to shore than its mother; the white edge of a cliff is in the foreground. In our experience, females always kept their calves inshore of them or interposed themselves between the calf and any potential danger — a boat, a swimmer, another whale, etc. In A, the slick in the water above the female's tail indicates that she has made a single stroke with her tail. In B and C, the strange spinal flexures of the approaching whale are shown.

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ZOOLOGICA

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NEW YORK ZOOLOGICAL SOCIETY

1

The Heliconians of Brazil (Lepidoptera: Nymphalidae). Part II.[1] Introduction and General Comments, with a Supplementary Revision of the Tribe.

(Plates I-VI; Text-figures 1-12; Map)

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The Lepidopterous tribe Heliconiini has recently become very useful in biological and genetic investigations. For this reason, it was important to obtain information on many previously little-studied species in the Amazon Basin and southern Brazil, and to clarify the systematics of the tribe in the light of this new information.

There are 18 species in the tribe normally found in extra-Amazonian Brazil, with seven more appearing marginally on the borders of the Amazon Basin. They demonstrate rather little variation or subspeciation over this area of nearly four-million square kilometers. In the mountains of the southeast, especially in subtropical areas, they undergo dramatic and cyclical annual variations in abundance, reaching their peak in late summer and fall (February through June).

The 37 species of Heliconiini found over the four-and-a-half million square kilometers of Amazonian Brazil are often very variable, and in many cases broken into multiple subspecies which often show parallelism in color-pattern with those of other species throughout the area; the subspecific divisions closely follow those observed in other animal groups and ascribed to Pleistocene weather changes. Many Amazonian heliconians demonstrate striking polymorphisms in single populations.

In light of new biological and distribution data, and with relation to Emsley's systematic revisions (*Zoologica*, 1963-1965), a total of 12 good species must be added to the tribe, and a further two must be recombined; the total number of species is now 66. A few remaining systematic uncertainties still remain, which could modify this number by two or three.

INTRODUCTION

THE LEPIDOPTEROUS TRIBE Heliconiini (Nymphalidae: Nymphalinae; also frequently referred to as a subfamily, Heliconiinae) has recently attracted much attention as a convenient and varied tool for biological and genetic investigations (M. G. Emsley, 19th Annual Meeting of the Lepidopterists' Society, Washington, D.C., June 15-18, 1968). A long series of papers has been published by the Department of Tropical Research of the New York Zoological Society on many aspects of heliconian life, taxonomy, mimicry, behavior, physiology, and genetics: Alexander, 1961a, 1961b; Baust, 1967; Beebe, 1955; Beebe, Crane, and Fleming, 1960; Brower, Brower, and Collins, 1963; Crane, 1954, 1955, 1957; Crane and Fleming, 1953; Emsley, 1963, 1964, 1965, 1970; Fleming, 1960; Sheppard, 1963; Swihart, 1963, 1964, 1965, 1967a, 1967b, 1968; Turner,

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[1] Part I of this series: see K. Brown, 1970. Part III: see following paper.

[2] Contribution No. 260 from the Departamento de Zoologia, Universidade Federal do Paraná.

1968a, 1971; and Turner and Crane, 1962. These papers have resulted in a thorough knowledge of the 14 heliconian species normally present in Trinidad.

We have recently initiated biochemical studies on Brazilian heliconians (K. Brown, 1965, 1967; K. Brown and Domingues, 1970; Tokuyama *et al.,* 1967) and thus have had to develop a similarly thorough knowledge of the species present in this area. We succeeded in delineating food-plants and observing at-least some part of the early stages of all 18 heliconian species normally present in extra-Amazonian Brazil (Appendix I). Most species proved to be readily bred in captivity and reasonably resistant to disease, parasitism, and handling. A few species, however, were not tractable even under the most ideal conditions. These included the four high-flying and uncommon-to-very-rare species, *Philaethria wernickei, Eueides pavana, Heliconius nattereri,* and *H. silvana ethra.* Partial to complete information on early stages, either observed in nature or reared from fertile eggs expressed from wild-caught females, and food-plants is nonetheless available for these species (Appendix I).

This paper presents a general view of the tribe in Brazil, with detailed comments on various species. It includes some specific corrections and additions to Emsley's recent papers (1963, 1964, 1965) on the Heliconiini, including comments on non-Brazilian species, and thus amounts to a complete supplementary revision of the tribe. The paper also contains a complete synopsis of the species occurring in extra-Amazonian Brazil and preliminary food plant data for them, as well as a brief list of the heliconians of the Brazilian Amazon. Part III includes a description of the biology of the key primitive species *Heliconius nattereri,* and a graphical formulation of the possible geohistorical evolution of the genera *Heliconius* and *Eueides.* Three new subspecies from the central Brazil plateau (Appendix I) will be fully described in Part IV. Part V is a revision and discussion of the mimetic silvaniforms (the first half of Emsley's *"numatus-*group"); its taxonomic conclusions are incorporated into the nomenclature used in this part.

TAXONOMY

The taxonomy of the Heliconiini was revised by Emsley in 1963-1965, reducing the recognized number of species in the tribe from 116 to 55. Useful papers have since been published by Turner (1966, 1967b, 1967c), clarifying the systematic positions and variations of *Heliconius demeter* and *H. elevatus,* and the nomenclature of *Dryas iulia.* Emsley's papers require a few

further clarifications, corrections, and additions in light of new information about Brazilian and extra-Brazilian heliconians, which brings the total number of recognized species in the tribe back up to 66 (see below and Appendix III). We also will separate the genus *Eueides* from *Heliconius* on morphological, biological, karyological, behavioral, and chemical grounds.

Emsley (1965) revised, where appropriate, the endings of all names in *Heliconius* and *Eueides* to masculine gender or genitive case. The inadvisability of such modifications of the endings of originally described specific names to agree with the supposed gender of an often changeable genus has been defended by Turner (1967d), with specific reference to the heliconians. His comment on the modification of *vesta* to *vestus* ("Scandal in Temple. Vestal Virgins say *We are just good friends"*) is truly classic and defends our preference, followed in this series of papers, for leaving all names as originally proposed by their authors.

VARIATION

One of the principal reasons that heliconians have been so useful to biologists is that they vary extremely in bright and colorful wing-patterns. The perfectly parallel variation of the common species, *Heliconius erato* and *H. melpomene,* over essentially all of tropical America and through at least 20 distinct basic color-patterns and over 200 named forms (Emsley, 1964; Turner, 1970), is material to astonish the layman, confound the collector, and delight the geneticist. The variability in *erato* and *melpomene* expresses itself most luxuriantly at the borders of the Amazon Basin (map, page 71). Here, the blue to black ground color, with one or two red forewing bands and often a yellow hind-wing stripe, typical of the extra-Amazonian forms of these two species, encounters the radically different Amazonian dennis-rayed pattern (Plate VI, figs. 63 and 64), also displayed by many other Amazonian *Heliconius* and *Eueides* species.[3] The complexity of forms occurring in a small area (northeastern Bolivia, central Ecuador, south-central Colombia, and French Guiana-Surinam are especially noteworthy) challenges the imagination. The transition zone between the Amazonian and extra-Amazonian color-patterns has yet to be thoroughly investigated in any part of Brazil, with the possible exception of the Obidos-Santarém area (Plate VI, fig. 64).

In central Mato Grosso, the start of pattern plasticity has been recorded in the literature (Talbot, 1928) and documented by us. Unusual variations of *erato* and *melpomene* frequently turn up there, along with several other species

normally confined to Amazonia and carrying the dennis-rayed pattern. However, at least 95 per cent of the populations are within normal limits of *H. erato phyllis* and *H. melpomene burchelli*. In June, 1971, apparently monomorphic populations of *H. erato phyllis* and of the dennis-rayed *H. e. venustus* and *H. melpomene penelope* were traced to within 100 Km of each other in western Mato Grosso, with no signs of hybridization being observed. By October, a wandering *phyllis* had apparently crossed the very inhospitable dory grassland between these colonies and introduced its genes into the northern *venustus* population, which showed over 30 percent of individuals with hybrid characters. In this area, however, the subspecies may be effectively separated by the grassland ridge of the Serra dos Parecis, unlike in lowland Bolivia where they hybridize extensively.

Two extra-Amazonian species (*Heliconius silvana ethra* and *H. ethilla narcaea*) show a moderate north-south variation that has resulted in the separation of weak but recognizable subspecies. Intergradation occurs, however, well into "typical" populations both north and south of any arbitrary boundary. These are the only two species that are appreciably polymorphic in extra-Amazonian Brazil. Four named forms

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[3] This pattern is apparent in the female of *Eueides vibilia unifasciatus*, in *E. eanes* and *tales*, and in *Heliconius aoede, burneyi, egeria, astraea, xanthocles, doris* (red forms), *elevatus, melpomene, timareta+* (except nominate form), *erato,* and *demeter* (species marked here with a cross are not known from the Brazilian Amazon and are in all cases marginally Amazonian, from higher elevations on the eastern slopes of the Andes). Four additional *Heliconius* species (*hierax+, himera+, clysonymus+,* and *ricini*) show a similar though simpler black-yellow-red pattern, which easily may be confused in the field with that of the dennis-rayed heliconians. *Eueides lampeto* and *isabella,* and the six species of silvaniform *Heliconius* [*ismenius+, silvana, numata* (= *aristiona*), *hecale* (= *quitalena*), *ethilla,* and *pardalinus*; see Part V for clarification of taxonomy], have a related black-yellow-orange pattern which suggests partial mimetic association with the dennis-rayed species (see Emsley, 1964: 281). Species in these two genera known from the Amazon Basin which do not show a black-yellow-red (or orange) pattern are almost all either orange with black bars and wing bands: *Eueides lybia, aliphera,* and *procula edias+,* and *Heliconius metharme, wallacei, hecuba+, heurippa+, luciana, cydno+, hermathena, telesiphe+, charitonia+, sara, leucadia, antiochus,* and *congener+*. See map, figures, and Appendix II.

of *silvana ethra* may be found in a single population in Espírito Santo, and six named forms plus dozens of minor individual variants of *ethilla narcaea* can be found in southern Minas Gerais. Appreciable variation is also evident in some populations of Amazonian heliconians marginal in central Mato Grosso (Appendix I, B and Plate VI). The total amount of polymorphic variation, however, is small, especially in relation to that observed in Amazonian populations, particularly silvaniforms (see Part V of this series).

ZOOGEOGRAPHY

While heliconians usually conform to major zoogeographic barriers, they are relatively strong flyers and we frequently have observed them crossing unfavorable terrain, ascending mountains, or apparently moving deliberately from one area to another. They are therefore not as useful as, say, the Ithomiinae for detection of finer zoogeographic boundaries (Fox, 1967).

In extra-Amazonian Brazil (map), the only barrier noticeably separating the heliconians is the divide formed by the high southeast coastal mountains, a geohistorically significant boundary affecting many groups of plants and animals. This restricts the tropical *Philaethria dido, Eueides vibilia, Heliconius melpomene nanna,* and *H. silvana ethra* and largely restricts *H. sara apseudes* to the coastal plains and foothill canyons north of Santa Catarina (where the high mountains are close to the ocean), and substantially restricts *Dione moneta* to the Paraná-Paraguay River Basin, Santa Catarina, and Rio Grande do Sul. The two subtropical species *Eueides pavana* and *Heliconius besckei* are native to the coastal and adjacent mountains which form this divide. Isolated and undifferentiated colonies of *besckei* (and of many other southeastern mountain butterfly species, some of which have evolved into recognizable subspecies) may be found in the Brasília area, northern Goiás, and isolated highlands in northeastern Brazil. The exceedingly rare and declining primitive species *Heliconius nattereri* (Parts I and III of this series) is confined to a very few select areas of undisturbed extensive virgin forest in eastern Brazil (Littoral-median region = northern Espírito Santo, eastern Bahia, and possibly eastern Minas Gerais, Alagôas, Sergipe, and Pernambuco). Other than the species mentioned above and marginal species (see below), the remaining nine heliconian species may be expected in nearly all parts of extra-Amazonian Brazil, with the exception of a very few high, cold, or excessively dry regions. Some species, notably *Dione juno, Dryadula phaetusa,* and *Philaethria wernickei,* while widespread, tend to be very strongly localized.

Altitudinal transitions seem to present only imperfect barriers to many heliconian species in extra-Amazonian Brazil, certainly with much less importance than as implied in Emsley (1964, 1965). *Agraulis vanillae maculosa* flies from sea level to the highest areas in southern Brazil (near 3000 meters). In the genus *Heliconius,* the Andean species *telesiphe* was mentioned by Emsley (1965) as the only species normally flying at elevations above 1300 meters. In southern Brazil, however, *H. besckei* is found from sea level (locally) to its mecca at 800 to 1600 meters. From there it ranges upward in summer to more than 2000 meters. In late summer, even *H. erato phyllis* can be found breeding at nearly 2000 meters elevation, many kilometers from the nearest valley below 1300 meters. Many additional *Heliconius* species (*hierax, hecuba, heurippa, timareta, cydno, himera,* and *clysonymus,* as well as some local races of *erato* and *melpomene*) also have been found by us and by other collectors in the 1300-2500 meter range on the Amazonian slopes of the Andes in Bolivia, Peru, Ecuador, and Colombia. However, it does seem that heights above 2500 meters are impassable for *Heliconius* other than *telesiphe* (though not for *Agraulis* or *Dione*).

Cyclic Annual Variations in Abundance

Many field observations suggest that most heliconians undergo great annual variations in abundance that are partially but not wholly related to extensive new growth on the passifloraceous foodplants, and possibly accompanied by appreciable range expansions. They commonly appear in late summer and fall in areas where they do not survive or are drastically reduced in numbers in winter. A similar pattern has been observed in the two North American subspecies of *Agraulis vanillae,* and has been suggested for *Dione moneta* in Texas (Gilbert, 1969).

Several areas of intermediate elevation have progressively increasing numbers of tropical heliconians from January (mid-summer) into late May or June, followed by disappearance (or great reduction) during the winter and spring. Areas at 600 to 1200 meters elevation in the coastal mountains, such as Curitiba (Paraná), Petrópolis (Rio de Janeiro), and Santa Teresa (Espírito Santo), provide good vantage points for observing this. In Curitiba, at 900 meters elevation, *Heliconius sara apseudes* and *H. erato phyllis* begin appearing only in summer; by early fall they are frequent, but they seem to disappear with the first winter frosts. Both species fly all year around in the neighboring lowland areas; no definite information is available suggesting seasonal diapause mechanisms in *Heliconius* species in Brazil. *H. silvana robigus* and *H. sara*

apseudes appear only in January and may be found only through May on the seaward slope of the coastal mountains in Petrópolis, an area 1000 meters in elevation and without winter frosts. In relatively warm Santa Teresa, at 600 to 800 meters elevation and above a rich tropical tableland area, *Eueides vibilia, Heliconius melpomene nanna, H. sara apseudes, H. silvana ethra,* and *H. nattereri* are encountered principally from January through June; they sometimes are very common in March and April, depending upon the year. These five species have actually been observed by K. B. moving up and down the seaward face of the mountains; and they appear first each summer at lower elevations near where larger streams run down the mountain-face.

The increased abundance of the mountain species *Eueides pavana* and *Heliconius besckei* at sea level near the foothills in winter, when they are not commonly encountered on the colder mountaintops, may result from a diminished upward movement of individuals from these populations during the cooler months.

The Chapada de Guimarães in central Mato Grosso seems to have an invasion of Amazonian species from lowland northern Mato Grosso in fall. The marginal species mentioned in the section below all have been found in this region rarely or not at all from September to February, commonly in May to July.

Finally, Prof. Dr. Heinz Ebert of Rio Claro, São Paulo, has observed that the southwestern species *Dione moneta* is common in central São Paulo in March to June, but very rare or absent there during the rest of the year. Whether local populations simply build up in fall, or the species invades from the south or west in response to unusual weather conditions or population pressure, has yet to be established.

A general discussion of the annual variation of butterfly frequencies in Brazil has also been published recently by Dr. Ebert (1970).

Marginal Species in Extra-Amazonian Brazil

The borders of the Amazon Basin in northeastern and central Brazil (map) are not well-marked zoogeographical barriers, being visible essentially as a gradual change from humid forest to more dry and open vegetation, and a number of normally Amazonian species of heliconian (Appendix I, B and II and Emsley, 1965) cross them into adjacent areas of the extra-Amazonian region.

Two areas have received special attention. One is the Cuiabá region of central Mato Grosso. Although in the basin of the Paraguay River, this region has a large influx of Ama-

zonian Lepidoptera, especially in the highlands ringing Cuiabá from the north (Rosário Oeste, Melguira, Serragem, Nobres, Tombador, Quebó) around through the northeast (Chapada de Guimarães, Buriti) to the east (São Vicente). Amazonian heliconians found by us to be marginal in this area include *Eueides vibilia unifasciatus, Heliconius sara thamar, H. melpomene burchelli, H. wallacei flavescens, H. ethilla* as a new subspecies (see Part IV of this series), *H. xanthocles melete,* and *Eueides isabella isabella.* The first three of these species also are found locally as far as the Paraná drainage in central and western Goiás, and *H. sara thamar* has been captured in the San Francisco River drainage in northwest Bahia (Rio Sapão; specimens in the Carnegie Museum).

In the northwesternmost tributaries of the Paraguay River in central-western Mato Grosso, between the swampy Pantanal around Cáceres and the high grassy ridge of the Serra dos Parecis, is an eastward extension of the north Bolivian rain forest, which is in turn contiguous with the general Amazonian forest (Hylaea) down the Rio Guaporé. Here the flora and fauna are very strongly Amazonian. In the heliconians, *H. erato* remains as the southern subspecies *phyllis;* but the Amazonian species *H. numata* (many variants), *H. silvana* as subspecies *mirus, H. aoede* as a new subspecies (Part IV), *H. burneyi,* and *H. leucadia* were all discovered in a few hours' collecting on the upper Rio Branco, a major tributary of the Rio Cabaçal, in June 1971. The last species was also found in the upper Rio Jaurú to the west, only a few kilometers from the upper Rio Guaporé, which flows to the Amazon within the Hylaea. Additional north Bolivian species which might be found with more intensive collecting in the Cabaçal-Jaurú area include *Philaethria dido* (known from coastal extra-Amazonian Brazil but not from the interior), *Eueides lybia* (possibly observed already on the Rio Branco), *Heliconius doris, H. hecale* (members of the *sisyphus* subspecies complex), and *H. elevatus perchlora.*

A further four species have been recorded as occurring in the "Cuyabá-Corumbá River System," a rather ill-defined area which may or may not be restricted to the Paraguay drainage in central Mato Grosso (not the Corumbá River in southern Goiás, however): *Heliconius ricini, H. astraea* as a new subspecies (Part IV) (one in the British Museum, *fide* J. R. G. Turner, and one in the Kaye collection, now part of the Allyn collection), *H. elevatus schmassmanni* (which may only be a variation of Neustetter's *aquilina*), and *H. demeter eratosignis* (for the last two, see Joicey and Talbot, 1925). We have

searched in essentially all habitats in central Mato Grosso without locating any of these species. They would not invade from the northwest where the lowland Hylaea extends into extra-Amazonian Brazil, since here they were not found south of the Pimenta Bueno area in southeastern Rondônia (map). In this region, still 400 Km north of the Cabaçal-Jaurú forests, *Heliconius erato* makes a transition from the dennis-rayed, open-forewing-banded form, *amazona,* to the reduced dennis-rayed, compact-forewing-banded form, *venustus* (Plate VI, fig. 63).

In view of the known parallelism of forewing band modifications in Amazonian dennis-rayed *Heliconius* (Appendix II), it would be expected that *demeter, astraea,* and *elevatus* would similarly acquire a compact square forewing yellow patch in areas adjoining the northwestern Paraguay Basin. Indeed, the Pimenta Bueno population of *elevatus* already shows about 50 per cent of the subspecies *perchlora* with this compact band. The four species may invade from the northeast into the extreme upper Rio Cuiabá, in a forested highland which gives birth also to five major Amazonian rivers, or into the northeastern Pantanal, where highly suitable lowland forest habitat exists. However, in northeastern Mato Grosso, the Hylaea is 400 Km north of the Cuiabá river system, connected with it only by sparse riparian forests very poor in Heliconiini. We are inclined to regard the specimens labelled "Cuyabá-Corumbá River System" as originating from northern Mato Grosso or eastern Rondônia; until these species are confirmed in extra-Amazonian Brazil, they will remain on the hypothetical list. Here we place also *H. antiochus,* which lives commonly in northeastern Mato Grosso, even well south of the Hylaea in typical *erato phyllis/melpomene burchelli* territory (the dry southeastern Amazon).

The second area is in northern Ceará, where a number of mountain ranges (Serras de Ibiapaba, Uruburetama, Maranguape, and Baturité) break the palm-grassland plain and provide oases for unusual butterfly life. Also included in this marginal region is the area of Dom Pedro in southern Maranhão, but not northwestern parts of Maranhão which are decidedly Amazonian. Dr. Dmitro Zajciw collected these areas in 1962-1963, and donated many of his specimens to the Museu Nacional in Rio. His material includes *Heliconius erato phyllis, H. melpomene burchelli, H. ethilla* near *eucoma,* and *H. ricini.* The last three are not known from further southeast along the Brazilian coast. Other species known from central Maranhão and probably present in the extra-Amazonian portion, which has not yet been visited by the

authors, are *Eueides lybia* and *i. isabella,* and *Heliconius doris, wallacei, burneyi, numata, sara thamar,* and *antiochus.*

Two Andean butterflies have been recorded in Misiones in Argentina (Hayward, 1951) and might eventually be found in western Paraná or Santa Catarina: *Heliconius numata* (= *aristiona,* see Part V of this series) *splendidus* and *Dione glycera.* To our knowledge, however, neither of these has yet been captured in Brazil.

SOME SPECIFIC COMMENTS ON THE SPECIES

Philaethria dido and *P. wernickei,* two large black-and-green species very similar on the dorsal wing surface, are clearly distinct on the ventral surface and have consistently different male genital valves (Text-figs. 1 and 2). Furthermore, they are sympatric at least over all of the lower and middle Amazon Basin (*wernickei* as subspecies *pygmalion*) and along the east coast of Brazil as far south as Rio de Janeiro (the Museu Nacional in Rio contains long series of both species from a number of localities). They may be readily distinguished, at times even in flight, by the under surface of the hindwing (Plate I, figs. 1 and 2): *dido* has a much redder ground-color (*wernickei* is black or gray), large silvered intervenal marginal spots (*wernickei* has only a series of faint submarginal whitish streaks), and a long white costal stripe limited by the subcostal vein (*wernickei* has a short white streak which drops below the vein and covers the black upper border of the green area). The flight of *dido* tends to be higher, more rapid, and less interrupted than that of *wernickei;* both species may be frequently encountered on hilltops. The mature larva of *wernickei* is much

more deeply and richly colored than that of *dido* (Beebe, Crane, and Fleming, 1960), with a dark brick-red head and prolegs (orange in *dido*); the pupae of the two species are nearly identical.

Agraulis lucina (Felder), a singular form from the upper Amazon and Andean slopes, should be separated from *A. vanillae,* with which it has been treated as conspecific in the past. The former possesses a dramatically different color-pattern on both wing surfaces and its wings are of a distinctly different shape from those of *A. vanillae* (Plate I, figs. 3 and 4). The two species occur sympatrically in much of the Brazilian upper Amazon and on the Andean slopes of Peru, Ecuador, Bolivia, and Colombia.

In the areas where they commonly fly together, occasional specimens of *vanillae* (*catella* Stichel) show a coagulation of the dark markings on the dorsal wing surface and a reduction of the silvering on the ventral surface, looking thereby somewhat like *lucina* (Plate I, figs. 3 and 4). However, *catella* retains the light forewing apex, broader hindwing, and dark distal spot in hindwing space Rs-M$_1$, all typical of *vanillae*. In these same areas, occasional specimens of *lucina* are more heavily silvered ventrally, adding to the impression that the two species intergrade. Indeed, they may well interbreed occasionally where they meet, though they occupy different ecological habitats; *vanillae* lives in open areas and second growth, while *lucina* lives in forest clearings.

The overall behavior of *lucina* in the field is closer to that of *Dione juno* or *D. moneta,* which it also mimics in color-pattern, than to

1   deeply notched   longer   strongly humped

2   shallowly notched   shorter   evenly curved   1.0 mm
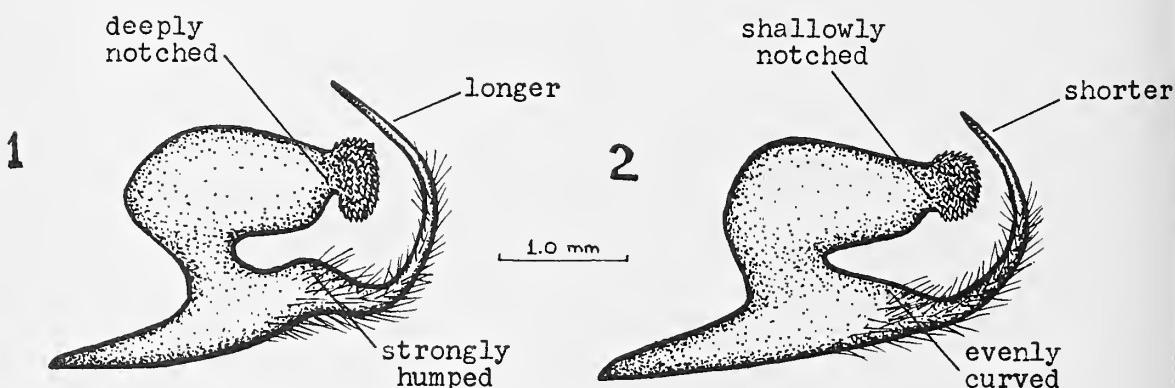
TEXT-FIGURE 1. *Philaethria dido,* male, left genital valve, external.
TEXT-FIGURE 2. *Philaethria wernickei* or *P. w. pygmalion,* male, left genital valve, external.

that of *vanillae*. The egg of *lucina*, expressed with difficulty from the female, is noticeably smaller and more spherical than that of *vanillae*, and a number of expressed eggs failed to hatch. This suggests that the eggs of *lucina* may be laid in clusters, as are those of *juno* (but not those of *vanillae*). Male genitalia of *lucina* could be consistently distinguished from those of *vanillae* (including *catella*) by the form of the process on the inner face of the valve. In *lucina*, the upper flange of the process is flared upward and narrowly serrated, while the lower posterior edge bears no teeth. In *vanillae*, the upper flange is curved inward and heavily serrated, and the lower posterior edge is usually denticulate (Text-figs. 3 and 4).

Eueides pavana, poorly represented in museums, is not at all rare in southeastern Brazil, occurring frequently in all the mountain area and sparsely down foothill canyons to the outwash plains at sea level. Its tendency towards high flight makes it somewhat difficult to capture.

Two color phases of the female exist, evidently in nearly equal numbers. One resembles the orange male, and the other is a pale straw color. Intermediates with the forewing light-colored and part or all of the hindwing orange (Plate I, fig. 5) also may be encountered. The species is sympatric with *Eueides vibilia* over much of the low-altitude part of its range (up to 800 meters, including the city of Rio de Janeiro); the dimorphic female of *vibilia* is quite similar in appearance to *pavana*, and both participate in the mimetic complex of *Actinote* spp. (distasteful Acraeinae), being almost indistinguishable from these on the wing (Plate I, fig. 5).

Eueides vibilia is also sympatric with the morphologically very similar *Eueides lampeto* in the Guianas (*l. copiosus*, Plate I, fig. 6), the Brazilian Amazon (*l. copiosus* and *l. lampeto*), and at various points on the eastern slopes of the Andes. As both species are quite localized and not frequently taken by commercial collectors, their micro-sympatry is not easy to prove. The two differ appreciably in size (*lampeto* is appreciably larger), wing-shape, and color-pattern. All races of *lampeto* demonstrate a large diffuse dark spot at the inner angle and an inward-directed dark triangle at the margin of forewing space Cu_1-Cu_2 (a wing area very useful in taxonomy both in heliconians and ithomiines) and heavy dark intervenal postcellular marks on the hindwing, lacking in *vibilia*. No intermediates are known from areas where the species fly near each other. The egg of *E. lampeto carbo* (Coroico, Bolivia, 1600 m) was totally dissimilar to that of *E. vibilia unifasciatus* from nearby Mato Grosso and Rondônia. The egg of *lampeto* is larger, creamy yellow instead of white with a pink cap as in *vibilia*, closely resembles that of *E. isabella*, and is laid singly rather than in large rafts as in *vibilia*. The appearance and individual feeding-pattern of the solitary first-stage larva of *lampeto* are very similar to those of gregarious *vibilia* larvae; both may be distinguished by the light-colored rather than black head from all other known first-stage *Eueides* larvae except *E. aliphera*. Thus, we regard *lampeto* and *vibilia* as closely related but distinct species.
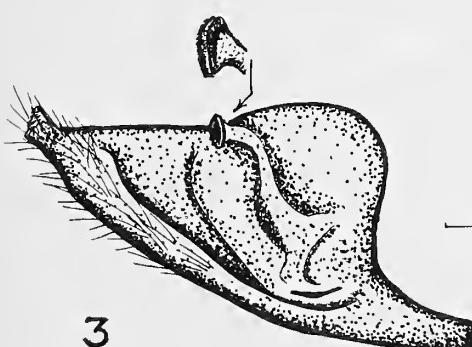
TEXT-FIGURE 3. *Agraulis vanillae,* male, left genital valve, internal, and detail of upper flange of process.
TEXT-FIGURE 4. *Agraulis lucina,* male, left genital valve, internal, and detail of upper flange of process.
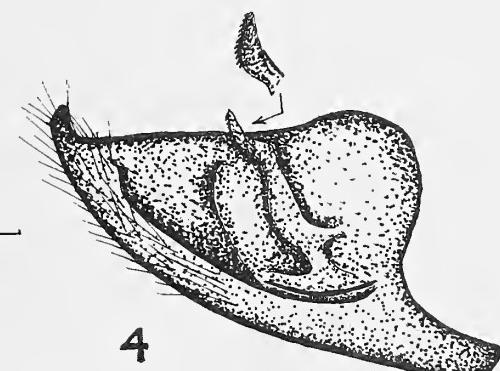
Heliconius egeria and *H. astraea,* represented in long series in the Museu Nacional, Rio, consistently show great differences in the male genital-valves (Text-figs. 5 and 6; see also Emsley, 1965), and usually in wing-shape and color-pattern. They apparently are sympatric at least at São Paulo de Olivença on the upper Amazon and at Manicoré on the Rio Madeira, where they do not intergrade morphologically, and so probably should be treated as separate species. Some middle and upper Amazonian and Venezuelan *egeria* (*e. hyas* Weymer) look very much like south-middle Amazonian *astraea* (which lack a name, having been assigned to *e. hyas* in the past; see Part IV for description) (Plate III, fig. 11); *egeria* usually has a more pointed forewing, with the hindwing rays more abbreviated and vein Cu red (in *astraea,* always black). However, as the color-patterns of the two are quite variable, and sometimes closely approach each other where the species fly together, examination of the genitalia is advisable for positive identification.

It is worthy of note that the Amazonian dennis-rayed heliconians (*Eueides tales* and *eanes,* and *Heliconius aoede, burneyi, egeria, astraea, xanthocles, elevatus,* and *demeter*) may be found flying together with red-forewing-band extra-Amazonian subspecies of *erato* and *melpomene,* not only in central Mato Grosso (*e. phyllis* and *m. burchelli* with *aoede* new subsp., *burneyi, xanthocles melete,* and possibly *astraea* new subsp., *elevatus schmassmanni,* and

demeter eratosignis in the northern part of the state), but also in Peru (*e. favorinus* and *m. amaryllis* with *aoede cupidineus, xanthocles melior, burneyi huebneri,* and *elevatus pseudocupidineus,* and *Eueides tales calathus* at Tingo Maria), north-central Colombia (*e. guarica* and *m. melpomene* with *burneyi lindigii* and *xanthocles flavosia* and polymorphs, and *Eueides eanes* and *tales cognatus* above Villavicencio), and in northern Pará, southeastern Venezuela and adjacent Guyana (*e. hydara* and *m. melpomene* with *aoede* near *astydamia* and *a. aoede, b. burneyi* and *b. catherinae, e. egeria* and *e. hyas, x. xanthocles, x. vala,* and *x.* subsp. incog., *elevatus barii, e. tumatumari,* and *e. roraima,* and *demeter bouqueti* and *d. beebei,* and *Eueides tales surdus* in Obidos to Itacoatiara and northward, Bolivar and western Guyana, and coastal Surinam and Guyane; see also map, fig. 64, and Masters, 1969; many of these species are polymorphic in this area for dennis only/dennis-ray). Upper Amazonian subspecies of *xanthocles* (*x. melete, x. melittus, x. melior,* and *x. flavosia,* plus several additional named and unnamed morphs) are unusual in lacking the subapical yellow forewing band typical of the lower Amazonian and Orinocan *x. xanthocles* and *x. vala* (*x. paraplesius* and the southeast Venezuelan race, probably new, are intermediate, showing partial fusion of the two yellow bands). They also possess many of the minor characters (Emsley, 1965) of *H. aoede:* paired intervenal submarginal white spots, a longer an-
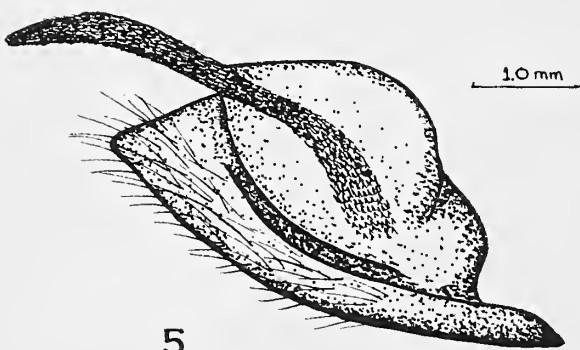
Text-figure 5. *Heliconius egeria,* male, left genital valve, internal.
Text-figure 6. *Heliconius astraea,* male, left genital valve, internal.
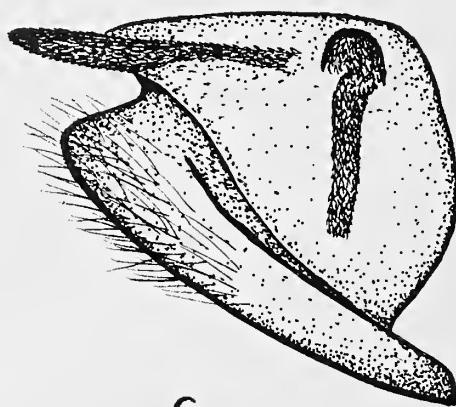
terior red basal spot, and a longer yellow costal stripe on the ventral surface of the hindwing; and prominent yellow lateral dots and intersegmental annuli on the abdomen. The males, however, have typical *xanthocles* genitalia and broad, rounded forewings, while sympatric subspecies of *aoede* (in southwestern Brazil, lowland Peru, and Venezuela) have the genitalia and deep triangular forewing, with an exceptionally broad androconial area on the costal half of the upper hindwing, typical of that species. Females in these areas may be quite difficult to distinguish.

In Mato Grosso, males of *xanthocles melete* have a rapid, fluttery flight, often quite high above the ground, and cover a large area in their promenading. The species is thus somewhat reminiscent of *H. nattereri* (see Part I and Part III), another primitive *Heliconius* occurring in extra-Amazonian Brazil, although in *xanthocles* the sexes are identical, though differing in flight habits.[4] It was not possible to express fertile eggs from the short abdomens of either *H. aoede* or *H. xanthocles* females, suggesting that the eggs may be laid all at once, and the caterpillars may be gregarious. This is so in *H. antiochus*, *H. sara*, and *Eueides vibilia*, other species with short abdomens, which give no fertile expressed eggs.

Some Notes on the *Melpomene*-group

Four species must be added to the *melpomene*-group as defined by Emsley in 1965 as part of the "*numatus*-group".[5] One of the two Brazilian species (*H. besckei*) was mentioned as probably distinct in Emsley's 1965 revision; later experiments have confirmed its specific status. The second species (*luciana*, which may be shown to be conspecific with *elevatus*), is unusual in that it escaped detection until 1960 (Lichy).

The tropical and subtropical habitat preferences of *H. melpomene* and *H. besckei*, respectively, in southern Brazil, do not often permit their occurrence at the same locality. Three areas where they have been found flying together are central Espírito Santo (in river valleys and up the mountain slopes to at least 1000 meters, principally in late summer), the foothill canyons near Rio de Janeiro (where *melpomene nanna* is very scarce), and the Brasília area in the central plateau (where *besckei* flies with *m. burchelli*). The two may also be found together over much of southern Mato Grosso and in northern Goiás and other isolated mountain areas in northeastern Brazil. Typical *besckei* also have been recorded as far west as Santa Cruz de la Sierra in Bolívia, where they fly with a polymorphic population of *melpomene* in

which the predominant subspecies *amandus* is partially infused with genes from the Amazonian *penelope*.

In all of these areas, no signs of intermediate characters have been found in many dozens of *melpomene* and *besckei* examined. Eggs of *besckei* from Petrópolis (1000 meters, near Rio de Janeiro) were bred through to adults on *Passiflora sidaefolia*. The egg, larva, and pupa were very similar to but distinct from those of *melpomene* (see Beebe, Crane, and Fleming, 1960). It should be noted, however, that these early stages are subject to much permissible variation, and that striking differences were observed by the first author in the size, color, and patterns of the eggs, larvae, and pupae of geographically separated but indubitably conspecific populations and subspecies of *Heliconius melpomene*, *H. erato*, *H. wallacei*, and many silvaniform *Heliconius*. Furthermore, male *besckei* showed no response to virgin females of *H. melpomene flagrans* in Trinidad (Emsley, 1970); indeed, they showed no reaction to any exclusively red-banded heliconians, but indulged in social chasing with red-and-yellow banded *H. erato phyllis* reared there from a previous shipment from Rio.

In life, *H. besckei* tends to have a higher and more fluttery flight, but also with more planing, than *H. melpomene*. The tip of the male genital valve in *besckei* is elongated, silvaniform rather than melpomeneform, similar to that observed in the closely allied and evidently allopatric species *H. elevatus* (Turner, 1967b). The ventral hindwing costal streak and basal spot complex are so different between *besckei* and *elevatus*, however, that it is highly unlikely that they could be conspecific. They may eventually be found flying together in central or southwestern Mato Grosso, meeting-ground of the Amazonian and southeastern forms of *melpomene* which these two species resemble in their respective ranges.

The existence of closely parallel species in the *melpomene* group and the *sara* group, *H. cydno* —*H. sapho*[6] and *H. pachinus*—*H. hewitsoni*, suggested the possibility of a *melpomene*-linked species parallel to the *sara*-linked *H. antiochus*. This species, *luciana* Lichy, was finally discovered in southern Venezuela in the late 1950s. A single female of *luciana*, from near Bôa Vista in the Brazilian territory of Roraima, on the southern slope of the Venezuelan highlands, is present in the collection of the Museu Nacional, Rio. The wing-pattern of this specimen is very similar to that of the sympatric and common *H. antiochus alba* on the dorsal surface. However, there are elements in common with *cydno* and *elevatus* (both in the *melpomene* group)

[4] A further presumably quite primitive heliconian species observed to have very similar large-scale promenading behavior is *Heliconius hecalesia formosus* in Panama.

[5] We have observed the complete sympatry of *Heliconius heurippa* with *H.m. melpomene* (Plate II, fig. 8) in the Rio Negro area above Villavicencio, Colombia, where they are among the 32 species of heliconian present (of a total of 51 so far recorded, with six more expected, in Colombia—a very high percentage of the 66 species in the tribe). We are grateful to Dr. E. W. Schmidt-Mumm and his brother Helmut of Bogotá for opportunities to visit the latter's property on the Rio Negro in 1969 and 1971. Here, *H. heurippa* and *H. melpomene* fly together in the altitude range 600 to 1600 meters, with *melpomene* found principally on the forest edge at lower elevations, and *heurippa* principally in clearings within the forest at higher elevations. However, they are frequently observed together. The eggs of *heurippa* (expressed from females) had more vertical ridges (17-18) than those of *melpomene*; the caterpillars and pupa, reared from these eggs showed many small but consistent differences from the corresponding early stages of *melpomene*. We judge *heurippa* to be a good "splinter species," probably originally arising from a yellow-banded ancestor of *melpomene* (see Emsley, 1964). The red outer band of *heurippa* is very inconspicuous in the field, and not likely to be useful in courtship recognition (see below, in text).

Heliconius cydno, a closely related species which also could be regarded as involved in the ancestry of *heurippa*, and which has nearly identical field behavior with the latter species, lacks the red basal dot pattern on the ventral surface of the hindwing shared by *melpomene* and *heurippa*, having in its place a variable U-shaped red-brown marking across the middle of the wing. *H. cydno* is absent from the restricted area where *heurippa* flies above Villavicencio, but could reach it, as have *H. erato guarica, m. melpomene,* and *charitonia bassleri,* presumably by going around to the north in southwestern Venezuela, or across several low passes in the southeastern Colombian cordillera. *H. cydno* in near-typical forms is definitely present on the southeastern slopes of the Venezuelan cordillera at Barinitas, and (together with another central valley species, *H. ismenius,* which has also been recorded near Villavicencio) on the Amazonian slopes of the eastern

Colombian cordillera above Florencia. In the central valleys of Colombia, the morphologically very close *cydno* and *melpomene* are common, fly together, and occasionally hybridize, producing little-known intermediate forms; these either strongly resemble *melpomene* (*rubellius, seitzi;* K. B. took one of these in Victoria, Caldas, on Jan. 21, 1971, in normal courtship with a female *melpomene,* not recognizing the hybrid until it was in the net), or have the double yellow-and-red forewing band as in *heurippa* and strongly resemble *cydno* in the field (*wernickei, emilius*). All of the hybrids have at least part of *cydno*'s U-shaped red-brown mark on the ventral hindwing (this is variable enough in the parent populations of *cydno* to admit its near absence in a hybrid, however); all show reduced but clear red basal spots intermediate between the three large dots of *melpomene* and the lack of spots of *cydno.* No hybrids are known to us from the *heurippa* area to the east of the eastern cordillera, and *heurippa* does not show hybrid characters other than the double-colored band.

The polymorphic yellow-banded species *Helicontius timareta* is completely sympatric, with no signs of intergradation, with the very different *H. melpomene plesseni* (and occasionally, with some of its intergrades to *H. m. aglaope*) in eastern Ecuador between 1000 and 1800 meters (Plate II, figs. 9 and 10). The field behavior of *timareta* is very similar to that of *heurippa* and *cydno;* it flies fairly high above the ground, and indulges extensively in repetitious promenading over set courses. It is found much more inside the steep pre-montane forest than is the sympatric *melpomene,* which prefers edges and riverbanks. *H. timareta* should be regarded as another distinct "splinter species," isolated at moderate elevations in the eastern Ecuadorian river valleys, and possibly closer systematically to *cydno* than to *melpomene.* Reproductive isolation from *melpomene* almost surely takes place by a color-courtship mechanism (see below, in text).

[6] The species regarded as *sapho* in Emsley (1965) is divisible into at least two fully sympatric species (Plate II, fig. 7) with dramatically different flight habits and behavior. One, represented by *sapho* and probably by *leuce,* occurs from southern Mexico to western Ecuador, flies high and slowly, frequently visits flowers, and is quite attached to one place both when feeding and when occupying a territory. The other, rep-

resented by *eleuchia* and *primularis,* and probably *eleusinus* and *ceres,* occurs from central Panama (Colon) to western Ecuador, and flies low, rapidly, and in a straight line, not stopping at flowers or remaining over long periods in one area. We are grateful to Dr. E. W. Schmidt-Mumm of Bogotá for detailed information on the sympatry and habits of these species in Colombia; we have fully confirmed his observations in Panama, Ecuador, and in museum collections. Dr. Tarsício Escalante of México also provided key information on the field behavior of *H. sapho leuce.* Where *sapho* and *eleuchia* fly together (Panama, central Colombia, and western Ecuador), they show no intergradation and rarely occupy the same habitats in the forest; in these areas, the latter species invariably has a shorter red costal streak and anterior red basal spot on the ventral surface of the hindwing, in relation to those of the former. Where only one form in the complex is known (*leuce* from México to Costa Rica, and *eleusinus* and its yellow morph *ceres* along the west coast of Colombia), this form shows the field behavior of *sapho* but the shorter red spots of *eleuchia.* Tentatively, *leuce* is placed with the former species; a short series from Nariño in extreme southwestern Colombia, present in the Instituto Oswaldo Cruz in Rio, strongly suggests intergradation of *primularis* with *eleusinus* through *ceres* and varieties; we thus tentatively place these three forms together with *eleuchia.*

A further very different-appearing and allopatric form which flies east of the Andes in Colombia, Ecuador, and northern Peru, *H. congener,* has the field habits and shortened red basal spots of *eleuchia.* If its reported chromosome number (33) is correct (de Lesse, 1967; presently being reconfirmed), it should stand as a good species. The allopatric *H. hewitsoni,* known only from the "Chiriqui" faunal region in southern Costa Rica and northwestern Panama, seems to merit its presently accepted specific status. There are thus most probably four species in the *sapho*-complex, apparently still in rapid evolution as the most recent major group in the Heliconiini.

Heliconius cydno also shows a separation into forms (*c. chioneus* and *c. cydnides*) which closely resemble *sapho* and *eleuchia* in Colombia. We have little field experience with the forms related to *cydnides,* and cannot completely eliminate the possibility that *cydno* may eventually be divisible into more than one species when more information becomes available, although this seems unlikely. In addition, *cydno* has some very unusual related forms in Colombia (*c. hermogenes* in the upper Magdalena valley, *c. weymeri* and its form *gustavi* in the

Cauca Valley) which do not resemble members of the *sapho*-complex, but approach other species of *Heliconius* (notably *hecalesia* and *erato chestertonii*) flying in the same areas. Kaye (1917) argued for the separation of these forms, which also frequently show a diminished or absent U-shaped red-brown mark on the ventral hindwing surface, from *cydno.* In defense of the unity of the species *cydno,* the following facts are presented: (1) *weymeri* and *gustavi* are evidently conspecific with *cydnides* and *cydno zelinde,* since complete intergradation among all of these forms is evident in series taken west of the cordillera northwest of Cali where low passes permit them to meet and mix (some forms illustrated in Holzinger & Holzinger, 1968); and (2) the intergradation of *c. cydno* and *hermogenes* can be seen in many intermediate specimens known from the middle Magdalena valley, and *hermogenes* apparently meets and intergrades with *weymeri* in select areas of the central cordillera. Thus, present evidence suggests the existence of but a single, if highly variable, species, *cydno,* in this complex.

An additional member of Emsley's *sara*-group, *H. hygiana,* is evidently interfertile with *H. clysonimus.* A polymorphic population exists northwest of Cali, Colombia, at high altitudes on the Pacific slope of the western cordillera, which includes occasional specimens of near-typical *clysonymus* and *hygiana,* a number of intermediates in color and pattern, and several unique endemic forms as well; morphologically, members of this population are nearer *hygiana,* but intermediate characters can be seen. It is probable that these two species, which have identical and quite singular field behavior among members of the genus, should be combined in spite of their appreciable morphological differences (Emsley, 1965; Holzinger and Holzinger, 1970). *H. hygiana* occurs from central-western Colombia through western Ecuador, at moderate to high elevations; *H. clysonimus* is known from similar elevations from Costa Rica to eastern Venezuela and southern Ecuador, but is sparse in central Colombia. It has been found on the inner face of the western cordillera near Cali, and in eastern Nariño; in these areas, where it can cross the western cordillera through passes below 2000 meters, it can meet and apparently occasionally interbreed with *hygiana.* The two are perhaps best regarded as "semispecies," very closely related in an evolutionary sense and not yet with perfect reproductive isolation in spite of long and nearly complete geographic isolation. For more details on the intermediate population northwest of Cali, see Holzinger and Holzinger, 1970.

on the ventral surface of the hindwing. In particular, there are a yellow streak under vein Sc-R$_1$ shared in the genus only by *elevatus,* and part of the unusual U-shaped red-brown bar of *cydno* (Text-fig. 7). This female was dissected; the bursa copulatrix has signa (Text-fig. 8) placing the species clearly within the *melpomene*-group (Emsley, 1965). The metapretarsus (Text-fig. 9) has paronychial processes nearly equal in length, and the abdominal processes (Text-fig. 10) are narrow, strongly curved at the base, and recurved near the outer tip, further confirming the placement of the species near *cydno* and *elevatus* in the *melpomene*-group.

In January 1970, K. B. examined the type-series of *luciana* (two pairs) in the Facultad de Agronomia, Universidad Central de Venezuela, Maracay, courtesy of Dr. Francisco Fernández Yépez of the Facultad. Although dissection of a male was not performed, the tip of the valve was examined under a 80x microscope and proved to be typically silvaniform, very similar to that of *H. elevatus,* but not like the abbreviated tip of the valve in *cydno.*

A most unusual series of *luciana* was taken by Mr. Harold Skinner of La Victoria, Venezuela, in April 1968 at Mantecal on the upper Rio Cuchivero in Bolivar, Venezuela, well north of the type-locality of the species. No two specimens of this series are alike; included are the typical white-banded form, a variety of yellow-banded forms with very variable forewing band shape and spots, and variable markings at the base of the hindwing, and one specimen which even has long yellow rays on the hindwing. We illustrate on Plate III (figs. 12-15), in addition to the type-series of *Heliconius luciana,* eight specimens from this series taken by Mr. Skinner.

In early 1970, a party of six collectors, including Mr. Skinner and Dr. Fernández Yépez, returned to Mantecal and captured a further fourteen *luciana,* all yellow-banded. According to Dr. Fernández Yépez, the species flies quite high above the ground and is difficult to capture except when it descends to flowers. Sr. Francisco Romero R., another member of the party, described the males as flying at more than ten meters of height above the ground, descending only occasionally to chase other passing *Heliconius* or species with similar flight or appearance.

In February 1970, K. B. was privileged to obtain through the kindness of Mr. Skinner a single male (Plate III, fig. 15) from the Mantecal series of *luciana.* The genital valves of this specimen (Text-fig. 11) are very close to those of *elevatus,* but show a somewhat less elongated
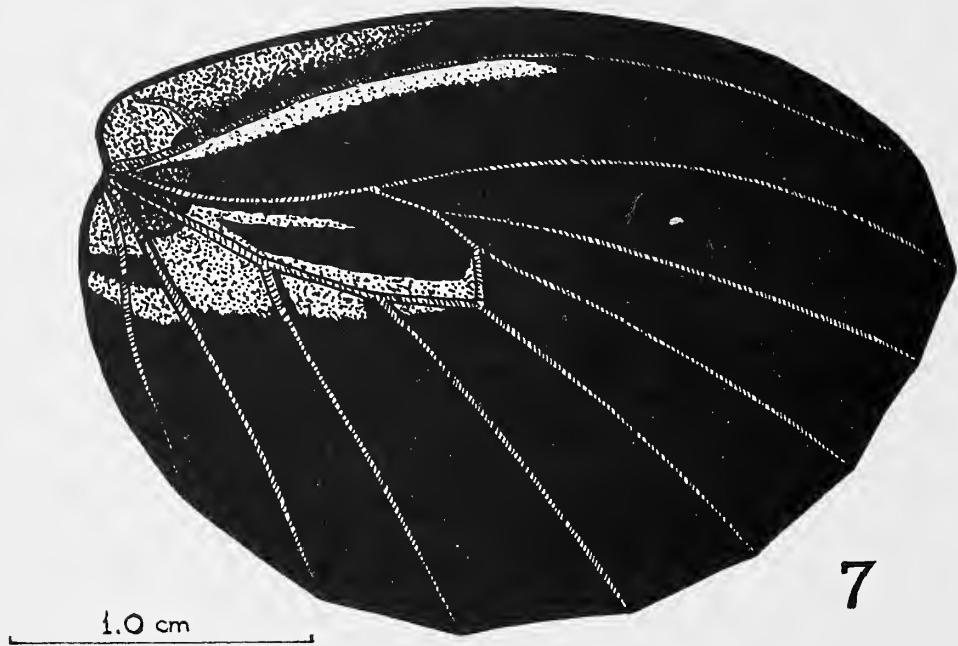
TEXT-FIGURE 7. *Heliconius luciana,* paratype female in the Museu Nacional, Rio de Janeiro, from Bôa Vista, Roraima, hindwing, ventral, schematic.
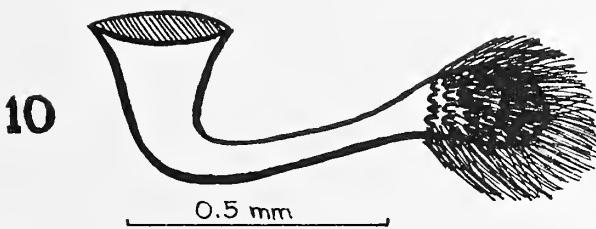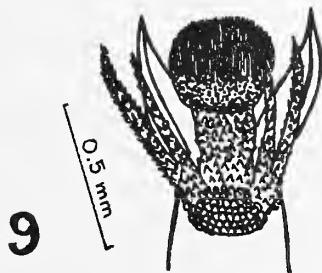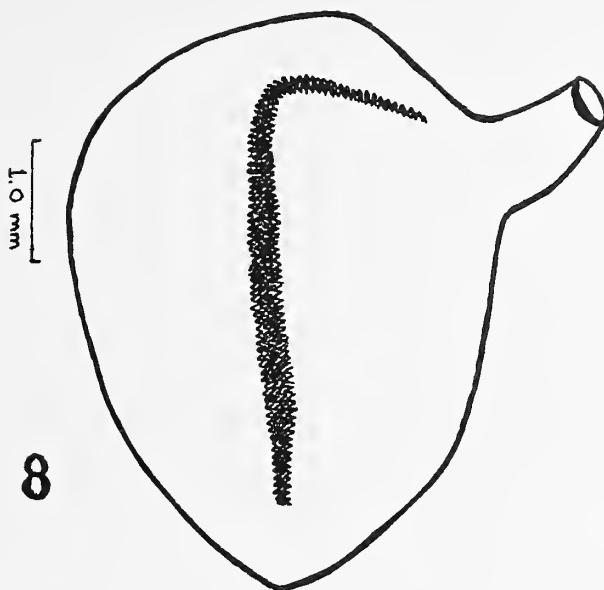
TEXT-FIGURE 8. *Heliconius luciana,* same female, bursa copulatrix.
TEXT-FIGURE 9. *Heliconius luciana,* same female, metapretarsus.
TEXT-FIGURE 10. *Heliconius luciana,* same female, abdominal process.

general form, a narrower dorsal process, and no apical tuft of bristles regarded as typical in *elevatus* genitalia (Turner, 1967b). However, as these characters have all been shown to be variable in the silvaniform valves, though the thickness of the dorsal process is usually reliable, we also compared female genitalia of the two species. The form of the signa on the bursae copulatrices is indistinguishable in the two, but *elevatus* possesses a vulvar plate markedly more lobed at the corners, and abdominal processes (Text-fig. 12) thicker, less curved at the base, and less recurved near the tip than those of *luciana*. These minor morphological differences between the two species are accompanied by significant differences in both major and minor elements of color-pattern between *luciana* and the nearest races of *elevatus* (*tumatumari* and *roraima*; Turner, 1967b, and Masters, 1969). *H. luciana,* in contrast to *elevatus,* possesses inclined rather than vertically arranged elements in the forewing subapical band; no dennis, but a yellow hindwing bar; usually a yellow stripe along the forewing cubitus; usually several red basal dots on the ventral hindwing; small or absent postcellular yellow elements on the forewing; and in most specimens a light submarginal spot in forewing space Cu_1-Cu_2, an element which we have found significant in correlation of the silvaniforms (see Part V). All of these facts lead us to regard *luciana* as specifically distinct from *elevatus.* However, at least one yellow-banded *luciana* has been taken at San Juan de Manapiare, 100 Km southwest of Mantecal (map), which could easily be interpreted as an intermediate between typical *H. luciana*

from farther south and *H. elevatus roraima* from farther east. Both species are presently so little-known that they have not been found flying in the same area; *luciana* has been found in Venezuela to the west of areas occupied by *elevatus,* and the latter species has not yet been captured in Roraima, Brazil. Thus, until more collecting in intermediate areas or interbreeding can be performed, we cannot completely eliminate the possibility of *luciana* being conspecific with *elevatus.* Further specimens of *luciana* captured in 1971 in central Venezuela and Bôa Vista conform to previous patterns, not casting new light on the problem; a population was discovered in Bolivar with equal representation of yellow and white-banded individuals.

It is of considerable interest that these five parallel species to *melpomene* within its same group (*H. timareta, heurippa, elevatus, luciana,* and *besckei*) apparently retain yellow as a courtship-release color, while red is distinctly the important color in *melpomene* (Emsley, 1964, 1970; Crane, 1957). The first three of the species have bright yellow forewing bands in all known forms, and maintain these, in the first with complete suppression of red (in the nominate form), in spite of being sympatric with red-banded forms of *melpomene.* The rare *luciana,* also sympatric with red-banded *erato* and *melpomene* in all its known localities, exists in yellow-banded and white-banded morphs. Either color is probably equally effective in courtship release, as they have similar reflectance and are interchangeable in many silvaniform heliconians such as *ismenius* and *hecale*
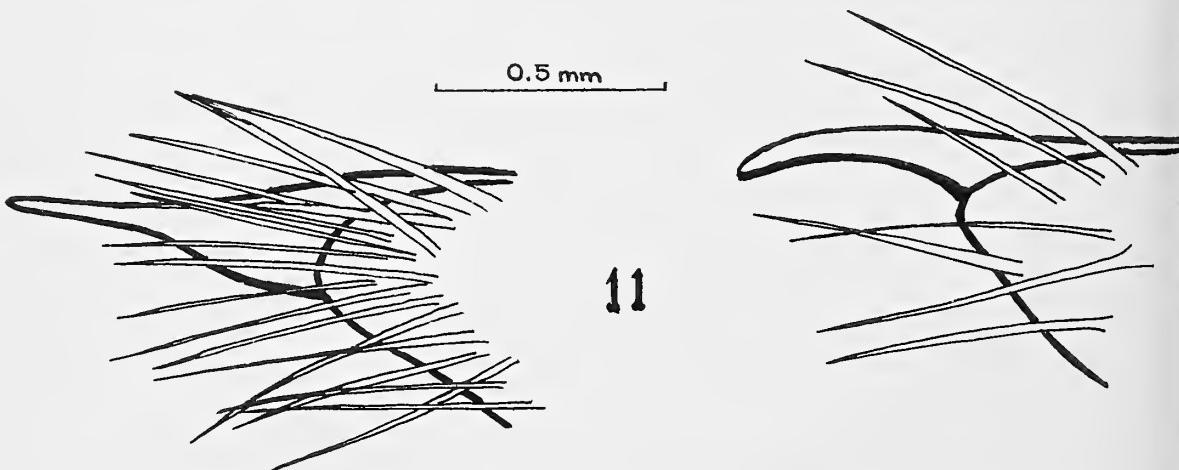
TEXT-FIGURE 11. *Heliconius luciana,* male from Mantecal, Rio Cuchivero, Bolivar, Venezuela, H. Skinner leg., tip of genital valve (at right), compared with valve tip of *Heliconius elevatus tumatumari* (at left), from north of Obidos, Pará (the latter has the dorsal process, normally curved inward toward the dorsal midline, straightened out for comparison).

(Part V). *H. besckei,* as mentioned above, seems to respond socially to yellow but not to red (Emsley, 1970). Yellow is presumably a more ancient color (Emsley, 1964), typical of the genus *Heliconius* and present in all of its members (chemical composition 3-hydroxy-L-kynurenine; K. Brown, 1967, and Brown and Domingues, 1970). This color predominates in the male of the most primitive *Heliconius, H. nattereri.* Thus, these five species parallel to *melpomene* may have been "left behind" in an evolutionary sense when *melpomene* appeared as a widespread and red-responding species, or they may have developed independently from the more primitive silvaniforms, which at least the last three resemble morphologically more than they do *melpomene.*

THE *Silvana*-GROUP IN EXTRA-AMAZONIAN BRAZIL

Our use of *silvana* and *ethilla* as species names, and the former as a group name for the silvaniforms of older authors, rests on data which is detailed in the fifth part of this series. With respect to the latter, a cross of *narcaea* from Rio de Janeiro with Trinidadian *ethilla* revealed good fertility in the offspring of the F_2 backcross to the latter, thus confirming their conspecificity as suggested by morphological studies (Emsley, 1965).

The polymorphism of *ethilla narcaea* in Rio was clarified by rearing eggs obtained from a female of the rare dark form *satis*; five adults, including three *narcaea* and two *satis,* were obtained from seven eggs. We thus believe that *satis* is merely a dark color-variant with a single gene or closely linked genes controlling its three constant color-pattern differences from *narcaea* (*N*-locus?—Turner, 1968 and 1971, and Sheppard, 1963).

In the cooler interior of Brazil, *narcaea* locally intergrades smoothly to the striking form *polychrous,* which has an excess of yellow on both wings, with almost complete suppression of orange. A very few areas are known where *polychrous* is nearly monomorphic, but it usually flies together with *narcaea* and interbreeds freely with it.

Kaye (1917) mentioned the existence of an unusual brand on the inner margin of the ventral surface of the forewing of male *robigus* and *ethra,* absent in Amazonian *silvana,* and on this basis, coupled with the extremely elongated genital valves of the first two forms, separated these from *silvana.* We have verified in the collection of the Museu Nacional that this brand is present in all *ethra* and *robigus,* and also, to a varying degree, in over half of all Amazonian *silvana.* The form of the genital valve in Amazonian *silvana* is also extremely variable, frequently being as elongate as those of the southern subspecies. Both the brand and the genitalia are variable characters in many subspecies of *H. numata, hecale,* and *ethilla.* The caterpillar and chrysalis of some *ethra* are noticeably different from those of Amazonian and Bolívian *silvana,* but these differences do not surpass those observed in geographically isolated subspecies of other *Heliconius.* In spite of the essentially complete geographical isolation of *ethra* from *silvana,* we regard the possibility of reproductive isolation between the two as very small, and thus maintain them for the present as a single species.

Recent breeding results have suggested that, in spite of many constant differences in pattern, behavior, and early stages, the silvaniform species *numata* and *silvana* may interbreed freely in some areas of the Amazon Basin, where they are sympatric and common over nearly six million square kilometers. In this paper, and until more extensive field and insectary experiments can be completed, the two species are still maintained as distinct.

12

0.5 mm
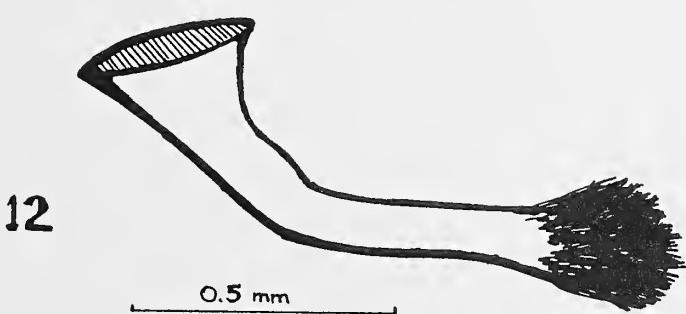
TEXT-FIGURE 12. *Heliconius elevatus tumatumari,* female, Obidos, abdominal process.

An Explanatory Note on Materials and Methods

We have based our conclusions on examination and study *in vitro*, with standard biological dissection methods, of all known heliconians, and extensive field experience with 59 of the 66 species recognized in the tribe. A biological rather than narrowly morphological definition of the species is advanced and, in the systematic ordering of these species, considerable weight has been placed upon *in loco* observations of adult behavior and micro-sympatry, and on characters of the early stages where these are known. Field observations and breeding were realized in Panama, Jamaica, Colombia, Venezuela, Trinidad, Guyana, Ecuador, Peru, Bolivia, and all areas of Brazil except the extreme northeast and the upper Rio Negro. Dr. Woodruff W. Benson also contributed additional field information from Costa Rica and Guyana. Complete Heliconiini collections were examined in the Museu Nacional in Rio de Janeiro; the Facultad de Agronomia in Maracay, Venezuela; the Allyn Collection (including the W. J. Kaye collection) in Sarasota, Florida; the Carnegie Museum in Pittsburgh, Pennsylvania; the Cornell University Entomology Department in Ithaca, New York; and the U.S. National Museum in Washington, D.C. Partially complete collections were studied of each of the authors, and of the Departamento de Zoologia in Curitiba, the Museu Goeldi in Belem, the Instituto Oswaldo Cruz in Rio, the Universidade Federal Rural of Rio de Janeiro, P. Gagarin and the late R. F. d'Almeida in Rio, L. W. Harris in Lima, E. W. Schmidt-Mumm in Bogotá, G. Small in the Canal Zone, F. Romero and H. Skinner in Venezuela, and W. Benson from Costa Rica and Guyana, among others. Further discussion of methodology is presented in Part III.

Summary

1. The taxonomy, variation, and zoogeography of heliconians are discussed, with particular reference to the 18 species regularly occurring in extra-Amazonian Brazil.

2. Significant cyclical annual variations in the abundance of species are noted, especially in the more subtropical areas and on the margins of the Amazon Basin; some possible mechanisms for these variations are discussed.

3. The following systematic changes are suggested in the tribe Heliconiini as defined and revised by Emsley (1963, 1964, 1965), based upon morphological study of museum specimens, field observation, breeding experiments, and proof of gross sympatry over large areas with or without evident intergradation:

a. *Philaethria wernickei,* and its Amazonian subspecies *P. w. pygmalion,* are separated from *P. dido.*

b. *Agraulis lucina* is separated from *A. vanillae*; the apparently transitional form *A. v. catella* may result from occasional hybridization, but appears to be true *vanillae.*

c. *Eueides lampeto* is separated from *E. vibilia*; the relationship of the latter to *E. pavana* is discussed.

d. *Heliconius astraea* is separated from *H. egeria*; where the two are sympatric, they are often nearly indistinguishable in color-pattern, but differ morphologically.

e. *Heliconius heurippa, H. timareta* and its forms, and *H. besckei* are separated from *H. melpomene.*

f. *Heliconius luciana* is added to the list of species, and fully discussed; its relationship to *H. elevatus,* still not perfectly defined, is explained.

g. *Heliconius eleuchia* with its subspecies *primularis,* and probably with *eleusinus* and its yellow morph *ceres,* are separated from *H. sapho. Heliconius congener* is also regarded as separate from *H. sapho.*

h. The species *H. hygiana* and *H. clysonymus* appear to be interfertile where they occasionally meet in western Colombia; the two are combined to form a single species *clysonymus,* though *hygiana* may be best regarded as a nearly isolated semispecies.

4. The probable use of yellow as a courtship-release color by five *Heliconius* species, closely parallel to the red-responding *H. melpomene,* is suggested.

5. A complete synopsis of known and hypothetical extra-Amazonian heliconian species in Brazil is presented, with behavioral and food-plant data and geographical distribution given for each species (Appendix I).

6. A brief synopsis of the heliconian species known from Amazonian Brazil is presented (Appendix II); the approximate divisions of the Amazonian subspecies of *erato* are defined, with indication of hybridization zones (map).

7. A brief summary of the systematic conclusions of this paper and of Part V (on the silvaniforms), which brings the number of species recognized in the tribe Heliconiini up to 66, is presented, with indication of the systematic problems still imperfectly resolved in the tribe (Appendix III).

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APPENDIX I

A Synopsis of the Heliconians of Extra-Amazonian Brazil

In polymorphic species, only significant color-morphs with well-established names are included in this synopsis.

Ranges are given for extra-Amazonian Brazil only. Abbreviations used for states are those standardized for use in Brazil: BA, Bahia; CE, Ceará; DF, Distrito Federal; ES, Espírito Santo; GO, Goiás; GB, Guanabara (city of Rio de Janeiro, formerly the Distrito Federal before the creation of Brasília); MA, Maranhão; MG, Minas Gerais; MT, Mato Grosso; PB, Paraíba; PR, Paraná; PE, Pernambuco; RJ, Rio de Janeiro; RS, Rio Grande do Sul; RN, Rio Grande do Norte; SC, Santa Catarina; SP, São Paulo. See map.

Indications of preferred flowers are: R = red (*Lantana, Gurania,* red *Bidens, Passiflora coccinea,* Poinsettia, Bromeliaceae); M = magenta (*Passiflora kermesina*); B = blue (*Stachytarpheta,* many *Eupatorium*); Y = yellow (*Oxypetalum,* yellow *Bidens,* many Compositae); W = white (many *Eupatorium,* Orchidaceae, Compositae).

The larval food-plants represent a very preliminary list. The passifloraceous species have been identified by the authors, by Dr. W. W. Benson, by Dr. C. M. Biezanko, and by Apparicio P. Duarte, following Killip's recent revision and Masters in Martius, *Flora Brasiliensis,* and more recent studies in Brazil, especially by Sacco; and by Dr. Stephen S. Tillett of Barquisimeto, Venezuela, following Killip and his own investigations. Tentative identifications are indicated with a question mark; several of the species used as food-plants by Brazilian heliconians are undoubtedly new. All the information on food-plants for Santa Catarina and Rio Grande do Sul is taken, with permission, from Biezanko, 1969. Food-plant records are based on field observations, usually on several occasions and in different areas, of feeding larvae and ovipositing females. For earlier works on the immature stages of Brazilian heliconians, see Turner (1967a).

A. NORMALLY EXTRA-AMAZONIAN HELICONIANS

Philaethria Billberg, 1820.

dido (Linné, 1763) (Plate I, fig. 1). Eastern coastal lowlands in forest at least from PB to RJ; not certain if present in the extreme south or in the interior (possibly marginal in central MT). Very localized and rather rare south of ES. Hilltops, though not strongly. Flowers W, Y, B, rarely R. Caterpillars solitary: *Passiflora mucronata* (ES); refused *P. alata, P. speciosa* (whose close relative *P. vitifolia* is accepted in Colombia and Panama), *P. violacea, P. jileki,* and *Tetrastylis ovalis* (ES).

wernickei wernickei (Röber, 1906) (Plate I, fig. 2, and Plate IV, fig. 16). Entire area in forest and on edges, commoner southward (but rare in coastal RS), very rare in central plateau except in central MT (where flies in cerrado near gallery forests); usually quite localized. Intergrades perceptibly to *w. pygmalion* (Fruhstorfer, 1912) northward. Hilltops. Flowers W, Y, B, R. Caterpillars solitary: *Passiflora sidaefolia* (GB, RJ), *P. coerulea* (SC, RS), *P. suberosa* (RS), *P. elegans* (RS), *P. mansii* (MT).

Dryadula Michener, 1942.

phaetusa (Linné, 1758) (Plate IV, figs. 20 and 21). Entire area in open country (fields, marshes, and scrublands), strongly localized but common where found. Flowers W, Y, R. Caterpillars solitary: *Passiflora mucronata* (GB), *P. misera* (BA), *P. mansii* (MT).

Agraulis Boisduval and LeConte, 1833.

vanillae (Linné, 1758) *maculosa* (Stichel, 1907) (Plate IV, figs. 17 and 18). Entire area, common to abundant, in open country only or in large cultivated areas within the forest. Flowers R, B, W, Y. Caterpillars solitary but tolerant: *Passiflora ichthyura* (ES), *P. mucronata* (GB), *P. edulis* (GB, BA, DF), *P. odontophylla* (?) (ES), *P. kermesina* (ES), *P. speciosa* (ES), *P. violacea* (ES), *P. quadrangularis* (GB, ES), *P. coerulea* (SC, RS), *P. mansii* (MT).

Dione Hübner, 1819.

juno juno (Cramer, 1779) (Plate IV, fig. 19). Entire area in forest clearings, but very rare in interior plateau; quite localized but common where encountered. Hilltops. Flowers R. Caterpillars strongly gregarious with coordinated behavior: *Passiflora edulis* (GB, RJ, BA), *P. alata* (GB, ES), *P. speciosa* (ES), *P. odontophylla* (?) (ES), *P. coerulea* (SC, RS).

juno suffumata Hayward, 1931. Isolated populations in the Brasília area; to be expected elsewhere in south-central Brazil (described from Paraguay). Both fore- and hindwings heavily suffused with black from margins inward; some specimens in populations in central MT tend towards this suffusion. Flowers R, B. Caterpillars gregarious: *Passiflora cornuta* (DF), *P. alata* (DF).

moneta moneta Hübner, 1825 (Plate IV, fig. 22). Southwestern MG, western SP and PR, and SC and RG, common in late summer and fall in open country and cleared areas within the forest. Perches in afternoon, and roosts at night on projecting tips of grass in open areas. Flowers R, W, Y. Caterpillars solitary: *Passiflora violacea* (SP).

Dryas Hübner, 1807.

juno juno (Cramer, 1779) (Plate IV, fig. 23 and 24). Entire area in all habitats, less common in interior plateau. Flowers R, W, Y, B. Caterpillars solitary and cannibalistic: *Passiflora organensis* (GB), *P. truncata* (GB, SP), *P. sidaefolia* (GB), *P. misera* (BA), *P. capsularis* (GB, SP), *P. quadrangularis* (RS), *P. coerulea* (RS), *P. edulis* (GB, RS).

Eueides Hübner, 1816.

aliphera aliphera (Godart, 1819) (Plate IV, fig. 28). Entire area, strongly localized, almost always encountered very near its food-plant. Flowers W, Y. Caterpillars solitary but tolerant: *Passiflora violacea* (GB, RJ, SP, MG, ES, BA, DF), *P. coerulea* (RS), *P. quadrangularis* (RS), *P. coccinea* (MT), *P. sidaefolia* (GB).

vibilia vibilia (Godart, 1819) (Plate I, fig. 5). Very local on coastal plain in deep forest, up to moderate elevations in the coastal mountains and river-valleys, south at least to PR. Observed in apparent unidirectional migration in late summer in ES, from the valley of the Rio Doce SE over the mountains towards the coast. Flowers W, Y, B. Caterpillars gregarious: *Passiflora odontophylla* (?) (ES).

pavana Ménétriés, 1857 (Plate I, fig. 5). Locally common in forest in the Serra do Mar and Serra da Mantiqueira at 600 to 1500 meters elevation, from central ES and central MG south to SC, also locally down to foothills and outwash plains at sea level. Flowers W, Y. Caterpillars solitary: *Passiflora sidaefolia* (RJ).

isabella (Cramer, 1781-2) *dianasa* (Hübner, 1806) (Plate IV, fig. 25). Entire area in forest, uncommon. Becomes plastic northward, showing an increasing percentage of yellow and/or divided subapical elements on the forewing (intergradation to *i. isabella*). Hilltops. Flowers W, Y, rarely R, B. Caterpillars solitary but tolerant: *Passiflora edulis* (GB, RJ), *P. alata* (GB), *P. odontophylla* (?) (ES).

Heliconius Kluk, 1802 (1780?).

nattereri C. and R. Felder, 1865 (illustrated in Part I and Part III of this series). Very rare and local in large tracts of virgin forest in the "Amazonian island" of lowland BA, ES, and possibly eastern MG. Prefers steep, humid areas where its foodplant is giving abundant fresh growth. Female =

fruhstorferi Riffarth, 1898. Flowers R, M, B, rarely W. Caterpillars solitary but tolerant: *Tetrastylis ovalis* (ES).

silvana (Cramer, 1781) *ethra* (Hübner, 1827-31) (Plate V, figs. 34-37). Coastal belt from PE south to central ES in deep primary forest, rarely up river valleys and foothill canyons to 900 meters elevation. Includes form *brasiliensis* Neustetter, 1907, and many additional minor varieties. Quite localized. Flowers R, W. Caterpillars solitary: *Tetrastylis ovalis* (BA).

silvana robigus Weymer, 1875 (Plate V, fig. 38). Coastal belt from southern BA (overlapping with *ethra*) to SC (occasional) in deep forest, rarely up to 900 meters elevation in foothill canyons, very rare and localized southward. Flowers R, W. Caterpillars solitary: *Passiflora alata* (GB), *P. sidaefolia* (GB), *P. rhamnifolia* (GB).

ethilla Godart, 1819 *narcaea* Godart, 1819 (Plate V, figs. 40-43, 45, 46). Entire area in many habitats (but prefers forest), north to southern BA and DF, northwest to eastern MT, and south to western RS. Form *satis* Weymer, 1884 (fig. 45) appears very rarely in all populations (commoner locally, up to five percent of populations, in ES, MG, RJ, and GB). Form *polychrous* C. and R. Felder, 1865 (figs. 43 and 46) is commoner in the central plateau and in SP, predominant in some populations westward. Ridgetops. Flowers R, M, B, rarely W, Y. Caterpillars solitary: *Passiflora sidaefolia* (GB), *P. alata* (GB, DF), *P. kermesina* (GB, ES), *P. jileki* (ES), *P. rhamnifolia* (GB), *P. nitida* (DF), *P. cornuta* (DF), *Tetrastylis ovalis* (ES).

ethilla flavomaculatus Weymer, 1894 (Plate V, fig. 39). Coastal belt from PB south to southern BA. Flowers R. Caterpillars solitary: *Passiflora recurva* (PE), *P. kermesina* (PE).

besckei Ménétriés, 1857 (Plate V, fig. 47). Mountains in forest above 700 meters elevation from central ES (possibly BA and PE, locally), northern GO, and southern MT (at lower elevations) south to western RS, also occasionally down foothill canyons to outwash plains at sea level. Flowers R, B, rarely W, Y. Caterpillars solitary to semi-gregarious, tolerant: *Passiflora sidaefolia* (RJ), *P. villosa* (RJ), *P. coerulea* (SC), *P. organensis* (RJ).

melpomene (Linné, 1758) *nanna* Stichel, 1899 (Plate V, fig. 48). Eastern coastal belt in forest from RN south to ES, rarely to GB and occasionally to SC, also up mountains and river valleys to 1000 meters

elevation. Flowers R. Caterpillars solitary: *Passiflora alata* (young, soft, shaded plants) (ES), *P. misera* (ES, BA), *P. violacea* (BA), *Tetrastylis ovalis* (ES).

erato (Linné, 1758) *phyllis* (Fabricius, 1775) (Plate V, figs. 50 and 51). Entire area in all habitats, common. Becomes plastic (including form *phyllides*) in central MT. Forms *artifex* and *cohaerens* appear normally in all populations. Adults roost communally at night, in groups of three to 20 individuals. Flowers R, B, rarely W, Y. Caterpillars solitary and cannibalistic: *Passiflora truncata* (GB), *P. organensis* (GB, RJ, ES), *P. jileki* (ES), *P. violacea* (BA), *P. misera* (ES), *P. sidaefolia* (GB), *P. alata* (SC, RS, but rejected in GB), *P. capsularis* (GB, ES, GO), *P. coerulea* (RS), *Tetrastylis ovalis* (ES).

sara (Fabricius, 1793) *apseudes* (Hübner, 1806) (Plate IV, fig. 32). Coastal plain in oceanside hammocks, forest, and second growth, from PB to SC, up mountains, common; very sparse in interior of São Paulo (Loreto) and MG (Belo Horizonte). Adults roost communally at night in groups of up to 40 individuals. Flowers W, Y, R, B. Caterpillars strongly gregarious: *Passiflora mucronata* (GB, ES), *P. sidaefolia* (GB), *P. rhamnifolia* (GB), *P. edulis* (RJ), *Tetrastylis ovalis* (ES).

B. MARGINALLY EXTRA-AMAZONIAN HELICONIANS

Eueides Hübner, 1816

vibilia (Godart, 1819) *unifasciatus* Butler, 1873 (Plate IV, figs. 26 and 27). Marginal, locally abundant in fall and early winter, in forest and scrub in central MT and southwestern GO. Populations include a few percent of *v. vibilia* and many intermediates. Flowers W, Y, B. Caterpillars gregarious with coordinated behavior: *Passiflora mansii* (MT).

isabella isabella (Cramer, 1781-2) (Plate VI, figs. 52-55). Marginal, local, in central MT; to be expected in MA, CE. Polymorphic. Flowers W, B, R.

Heliconius Kluk, 1802 (1780?)

aoede (Hübner, 1809-13) manuscript subspecies, K. Brown (see Part IV of this series). One specimen from the Rio Branco, tributary of the Rio Cabaçal (Paraguay drainage) in west-central MT, a male taken in the afternoon of a cloudy day flying together with very similar-appearing ithomiines in heavy riparian forest, 400 meters elevation. Flowers R, W. Caterpillars probably gregarious.

wallacei Reakirt, 1866 *flavescens* Weymer, 1890 (Plate IV, fig. 29). Marginal in forest in central MT, to be expected in MA. One record of form *parvimaculata* Riffarth, 1900 from SC may be a labeling error. Flowers R, W, B. Caterpillars semi-gregarious: *Passiflora coccinea* (MT); *wallacei* is closely associated with this species and its very close relatives throughout its range in the Amazon and Orinoco Basins.

burneyi (Hübner, 1827-31) near *burneyi* (Plate VI, fig. 60). One specimen known from Cáceres in west-central MT; one male observed for over 30 minutes on high yellow flowers on the Rio Branco, tributary of the Rio Cabaçal, in June 1971. To be expected elsewhere in central MT and in MA.

xanthocles Bates, 1862 *melete* C. and R. Felder, 1865 (Plate IV, fig. 30). Regular in fall and winter in forests by streams in highland central MT. Flowers R. Caterpillars probably gregarious.

silvana (Cramer, 1781) *mirus* Weymer, 1894 (Plate VI, fig. 59). Marginal, well-established in west-central Mato Grosso (Rio Branco/Rio Cabaçal, 400 meters).

numata (Cramer, 1780-82) *superioris* Butler, 1875 and many forms near this (Plate VI, figs. 56-58). Marginal, common in west-central MT (Rio Branco/Rio Cabaçal, 400 meters); to be expected elsewhere in central MT. Caterpillars solitary: *Passiflora coccinea* (MT), *P. glandulosa* (MT), accepted *P. tricuspis* (MT).

ethilla Godart, 1819 *eucoma* (Hübner, 1827-31) (Plate V, fig. 44). Marginal in western CE and southeastern MA (D. Zajciw); may appear in west-central MT (Rio Cabaçal).

ethilla manuscript subspecies, K. Brown (see Part IV). Marginal but regular, frequent in fall, in deep forest near streams in highland central MT. Males promenade in small clearings. Flowers R, B. Caterpillars solitary: *Passiflora cornuta* (MT), *P. glandulosa* (MT).

melpomene (Linné, 1758) *burchelli* Poulton, 1910 (Plate V, fig. 49). Borders of Amazon Basin, in forest and cerrado, in MA, CE, GO, DF, and MT; becomes plastic, occasionally even with dennis and ray, in central MT. Flowers R, B. Caterpillars solitary: *Passiflora cornuta* (DF), *P. mansii* (MT), probably *P. tricuspis* (MT).

ricini (Linné, 1758) (Plate IV, fig. 32). Marginal in MA and CE; possibly marginal but unlikely in central MT ("Cuyabá-Corumbá River System").

sara (Fabricius, 1793) *thamar* (Hübner, 1806) (Plate IV, fig. 33). Locally common in forest and second growth on the borders of the Amazon Basin in MT, central and southern GO, DF, and extreme northwestern BA (Rio Sapão). To be expected in MA and CE. Flowers W, Y, R, B. Caterpillars gregarious: *Passiflora mansii* (MT).

leucadia Bates, 1862 *pseudorhea* Staudinger, 1896 (Plate VI, fig. 61). Marginal in west-central MT (Rio Branco/Rio Cabaçal and upper Rio Jaurú).

C. Hypothetically Extra-Amazonian Heliconians

The following species are either tenuously recorded from extra-Amazonian Brazil, with no recent and reliable confirmation, or else occur commonly in the indicated areas adjacent to extra-Amazonian Brazil, or have been recently recorded from these areas by reliable authorities. None has yet been captured by the authors in the area under consideration.

Dione glycera (C. and R. Felder, 1861). Misiones, Argentina (Hayward, 1951).

Eueides lybia lybia (Fabricius, 1775). Maranhão, Rondônia; possibly seen on the Rio Branco, MT, in June 1971.

Heliconius astraea Staudinger, 1896 manuscript subspecies, K. Brown (see Part IV). Rondônia and northern MT; "Cuyabá-Corumbá River System."

Heliconius doris doris (Linné, 1771) and form *delila* (Hübner, 1813). Maranhão, northern Mato Grosso and Rondônia.

Heliconius numata (Cramer, 1780-82) *splendidus* Weymer, 1894. Misiones, Argentina (Hayward, 1951).

Heliconius hecale (Fabricius, 1775) *sisyphus* Salvin, 1871 and variants. Northern Bolivia west of the Rios Cabaçal and Jaurú.

Heliconius elevatus Nöldner, 1901 *schmassmanni* Joicey & Talbot, 1925 (? = *aquilina* Neustetter, 1925) and *H.e. perchlora* Joicey & Kaye, 1917. Rondônia and northern MT; "Cuyabá-Corumbá River System."

Heliconius demeter Staudinger, 1895 *eratosignis* Joicey and Talbot, 1925. Southeastern Rondônia; "Cuyabá-Corumbá River System."

Heliconius antiochus (Linné, 1767) *alba* Riffarth, 1900. Northeastern Mato Grosso (common), Maranhão.

APPENDIX II

A Brief List of the Heliconians of Amazonian Brazil

The Amazonian region of Brazil is so little-explored that it would be most premature and foolhardy to present a definitive list or a complete synopsis of the subspecies at this time. A large network of highways, now under construction and to be finished by the mid-1970s, will permit a far more thorough investigation of Amazonian heliconians by the end of this decade. This list presents only a preliminary tally of the species and ranges known to date, with indications of the principal subspecies present where these are reasonably well defined. Marginally Amazonian species like *besckei* are omitted.

Many Amazonian heliconian populations are noted for their polymorphism. This phenomenon is perhaps most marked in *Heliconius numata,* discussed in detail along with other Amazonian silvaniforms in Part V of this series. Some examples of polymorphism in Amazonian heliconians are illustrated on Plate VI.

The Amazonian area of the map has been divided and patterned according to present information on the interaction of the subspecies of *Heliconius erato* in the Amazon Basin. Relatively monomorphic areas are indicated by pure patterns, blend zones (see Plate VI, figs. 63 and 64) by overlapping patterns; much of the Brazilian upper Amazon is a blend zone for three major subspecies, and the named form *lativitta* Butler 1877 is a typical hybrid from this area which shows signs of influence of all three of these subspecies (*amazona, emma,* and the *reductimacula-donatia-venustus* complex). The various color-patterns of *erato* in the Amazonian area are closely followed by those of the other dennis-rayed heliconians (*Eueides tales* and *eanes, Heliconius aoede, burneyi, egeria, astraea, xanthocles, elevatus,* Amazonian *melpomene,* and *demeter*). However, some startling exceptions to this generalized parallelism are known, presumably due to individual differences in the genetic mechanisms by which each species achieves the desired patterns.

Where *erato* and *melpomene* are red-banded in the north-central and southeastern parts of the Amazonian basin, the other dennis-rayed species may exist in unchanged form. They also may be replaced by closely related species (like the substitution of *luciana* in the north and *besckei* in the south for *elevatus*), or may be absent (as in most of Amazonian Goiás). The definition of the blend areas necessarily is approximate until detailed studies can be made along the new roads. Evidence accumulated over 50 years also indicates that both the position and the composition of these hybrid zones is constantly changing, in dynamic equilibrium with the monomorphic zones which give rise to them and with natural selection phenomena which vary within them from year to year.

Philaethria dido (Linné, 1763) (Plate I, fig. 1). Entire area except dry southeastern Amazon (*erato phyllis* area), quite frequent in heavy forest and clearings; very high flyer.

Philaethria wernickei (Röber, 1906) *pygmalion* (Fruhstorfer, 1912) (see Plate I, fig. 2). Upper Rio Negro and Uaupés, and southern Rondônia eastward through entire middle and lower Amazon.

Dryadula phaetusa (Linné, 1758) (Plate IV, figs. 20-21). Entire area, localized in open or marshy areas.

Agraulis vanillae (Linné, 1758) (Plate IV, figs. 17 and 18; Plate I, figs. 3-4). Entire area, but rare and local westward where following species flies. Principally nominate subspecies, except in southern Amazon [*v. maculosa* (Stichel, 1909)].

Agraulis lucina Felder, 1862 (Plate I, figs. 3-4). Upper Amazon only, from Uaupés, Tefé, and eastern Acre westward, in forest clearings.

Dione juno juno (Cramer, 1779) (Plate IV, fig. 19). Entire area though quite localized, in forest clearings; southwestern populations show appreciable variation in dark markings.

Dryas iulia iulia (Fabricius, 1775) (Plate IV, figs. 23-24). Entire area, frequent in all habitats.

Eueides aliphera aliphera (Godart, 1819) (Plate IV, fig. 28). Entire area, very localized, common along streams.

Eueides vibilia (Godart, 1819) (Plate I, fig. 5; Plate IV, figs. 26-27). Nominate subspecies rarely encountered in lower and middle Amazon; *v. unifasciatus* Butler, 1873 locally common in upper Amazon. Intermediates with partial forewing subapical bands are common in populations of both subspecies in the Amazon Basin.

Eueides lampeto Bates, 1862 (Plate I, fig. 6). Nominate subspecies very local and rare in upper Rio Solimões (above Tefé); *l. copiosus*

Stichel, 1906 recently discovered north of Obidos.

Eueides eanes eanes Hewitson, 1861. Not rare in extreme western Amazonas and Acre.

Eueides isabella isabella (Cramer, 1781-2) (Plate VI, figs. 52-55). Entire area but quite local; strongly polymorphic, especially south-westward.

Eueides lybia lybia (Fabricius, 1775). Entire area but rarer westward and southward; local in dryer areas and secondary forest, always found very near its food-plant.

Eueides tales (Cramer, 1775-6). Locally common in forest and second growth; the rather variable subspecies *pythagoras* Kirby, 1900 (dennis-ray), *tales* and *surdus* Stichel, 1903 (dennis only), *aquilifer* Stichel, 1903 (condensed FW yellow patch), and *calathus* Stichel, 1902 (FW yellow band distal to cell) follow the *erato* variations indicated in map. Not known outside the Hylaea in the dryer southeastern Amazon (Goiás).

Heliconius metharme (Erichson, 1848). Very local, from western Pará northwestward and southwestward to Uaupés, Benjamin Constant and western Acre.

Heliconius aoede (Hübner, 1809-13). Entire area except dryer southeastern Amazon. Subspecies *aoede* (dennis-ray), *astydamia* (Erichson, 1848) (dennis only), *faleria* Fruhstorfer, 1910 (partially coagulated FW yellow band), *lucretius* Weymer, 1890 (condensed FW yellow patch) and a new subspecies with reduced dennis (see Part IV), and *bartletti* Druce, 1876 (FW yellow band distal to cell) closely follow *erato* variations (Map I). Generally uncommon and local, in heavy moist forest.

Heliconius wallacei Reakirt, 1866 (Plate IV, fig. 29; Plate VI, fig. 62). Entire area, common wherever *Passiflora coccinea* and related species grow, many habitats. Usually *flavescens* Weymer, 1890; white-banded forms [*clytia* (Cramer, 1775-6) and *elsa* Riffarth, 1899] and *w. wallacei* are more frequent in the northern Amazon; polymorphic populations (*colon* Weymer, 1890; *parvimaculata* Riffarth, 1900, and many other forms) occur in the lower middle Amazon.

Heliconius burneyi (Hübner, 1827-31) (Plate VI, fig. 60). Entire area except extreme southeast, rather localized; very high flyer. Subspecies *burneyi* (dennis-ray), *catherinae* Staudinger, 1885-8 (dennis only), *ada* Neustetter, 1925 (partly coagulated FW yellow band with reduced subapical elements), and *huebneri* Staudinger, 1896 (condensed and reduced FW yellow patch and wider HW

rays) occupy areas roughly corresponding to *erato* variations, though much discrepancy from parallelism is seen and the overall variation of *burneyi* is less (see Map I).

Heliconius egeria (Cramer, 1775-6) (Plate III, fig. 11). Rare and local, from Belém west in heavy forest to Uaupés, western Amazonas and northern Rondônia; very high-flyer. The rayed subspecies *hyas* Weymer, 1884 is a variable element in many populations, predominant in the Rio Madeira region; its northern form with a more compact FW band, *asterope* Zikán, 1937, is found on the upper Rio Negro.

Heliconius astraea Staudinger, 1896 (Plate III, fig. 11). Rare and local in southwestern and extreme western Amazon, in heavy forest or along rivers; habits as in *egeria* and *burneyi*. Nominate subspecies in western Amazonas above Tefé; new subspecies (see Part IV) in the Rio Madeira area, south to well beyond the range of *egeria hyas*.

Heliconius xanthocles Bates, 1862 (Plate IV, fig. 30). Entire area except extreme southeastern Amazon. Subspecies *vala* Staudinger, 1885-8 (dennis-ray), *xanthocles* (dennis only), *paraplesius* Bates, 1867 (partly coagulated FW yellow band), *melete* Felder, 1865 (condensed FW yellow patch), and *melittus* Staudinger, 1896 (FW band distal to cell) closely follow *erato* variations (Map I).

Heliconius doris (Linné, 1771). Entire area, principally along major rivers. Forms *delila* (Hübner, 1813), *metharmina* Staudinger, 1896, and *amathusius* (Cramer, 1777) occur in all populations, but are commonest in far western and southwestern Amazon. Essentially no green forms have been found in the Brazilian part of the Amazon Basin. This species has been placed by recent authors in a separate subgenus (*Laparus* Billberg, 1820).

Heliconius silvana (Cramer, 1781) (Plate VI, fig. 59; Plate V, figs. 34-37). Entire area except southeast Amazon, quite common. Grades smoothly into subspecies *mirus* Weymer, 1894 in southern Rondônia; some eastern (Belém) specimens seem to grade towards *ethra* (Hübner 1827-31); far western specimens have larger subapical spots on the FW (as does the sympatric *numata aurora*).

Heliconius numata (Cramer, 1780-2) (Plate VI, figs. 56-58). Entire area except extreme southeast, locally abundant, highly polymorphic. Forms which may deserve weak subspecific rank include *superioris* Butler, 1875 (middle Amazon southward), *aurora* Bates, 1862 (far west), *euphone* Felder, 1862 (south-

west), and *zobrysi* Fruhstorfer, 1910 (southeast); *silvaniformis* Joicey and Kaye, 1917 is a strong element in far eastern populations. Some southwestern specimens have a black suffusion on the distal half of the FW as in *silvana mirus*. Dissimilar specimens in which the yellow has been entirely replaced by orange occur in all populations; the extreme of these is *arcuella* Druce, 1874, commoner westward; the orange form of *superioris* is *isabellinus* Bates, 1862; of *zobrysi* is *seraphion* Weymer, 1894. The very dark hindwing of nominate *numata* appears in all populations, but is commoner northeastward.

Heliconius ethilla Godart, 1819 (Plate V, figs. 39-46). The subspecies *eucoma* (Hübner, 1827-31) and its dark variety *numismaticus* Weymer, 1894 occupy almost the entire area, except for the southeast (*e. narcaea* Godart, 1819 and its form *polychrous* Felder, 1865), southwest (*nebulosa* Kaye, 1916), and south-central Amazon (new subspecies, see Part IV). The Guianian subspecies *thielei* Riffarth, 1900 may appear in the northeastern and north-central parts of the Amazon Basin.

Heliconius hecale (Fabricius, 1775). Entire area except southeastern and south-central Amazon. Strongly fragmented into locally differentiated populations with apparently rather limited gene-flow, which may be regarded as good subspecies: *novatus* Bates, 1867 (Belém; erroneously rechristened *schulzi*); *xinguensis* Neustetter, 1925 (lower Rio Xingú); *paraensis* and *latus* Riffarth, 1900 (Obidos area); *vetustus* Butler, 1873 (north of Obidos into Guianian highlands); *metellus* Weymer, 1894 (near Santarém); *fortunatus* Weymer, 1884 (north of Manaus); *spurius* Weymer, 1894 (south and east of Manaus, a minor element as far east as eastern Pará); *sulphureus* Weymer, 1894 (Rio Negro); *ennius* Weymer, 1890 (south and west of Manaus); *nigrofasciatus* Weymer, 1894 (Rondônia and Acre); *sisyphus* Salvin, 1871 and forms *concors, jonas*, etc., Weymer, 1894 (extreme west and southwest); *humboldti* Neustetter, 1928 (extreme west north of the Solimões), and probably many more to be discovered.

Heliconius pardalinus Bates, 1862. Principally from extreme western Amazonas (*pardalinus*) east to Rondônia and Manaus (form *lucescens* Weymer, 1894 commoner), possibly to Obidos and Santarém; also southwest to Acre (*maeon* Weymer, 1890 and *dilatus* Weymer, 1894).

Heliconius elevatus Nöldner, 1862. Entire area except southeast and north-central Amazon, but extremely rare and localized. Subspecies *barii* Oberthür, 1902 (dennis-ray), *roraima* Turner, 1967 and *tumatumari* Kaye, 1906 (dennis only, the former with a condensed FW yellow patch), *aquilina* Neustetter, 1925 and (or=?) *schnassmanni* Joicey and Talbot, 1925 (partly coagulated FW yellow elements), *perchlora* Joicey and Kaye, 1917 (condensed FW yellow patch), and *elevatus* (FW band mostly distal to cell) follow fairly well the divisions of *erato* (see Map I).

Heliconius luciana Lichy, 1960 (Plate III, figs. 12-15). Known only from near Bôa Vista in northern Roraima, where sympatric with the very similar and abundant *antiochus* and uncommon *wallacei elsa*.

Heliconius melpomene (Linné, 1758) (Plate II, figs. 8 and 10; Plate V, fig. 49). Entire Amazon Basin, locally abundant but often absent from large areas. Subspecies *thelxiope* (Hübner, 1806) (dennis-ray), *meriana* Turner, 1967 (dennis only), *madeira* Riley, 1919 (partly coagulated FW yellow band), *vicina* Ménétriés, 1857 (condensed FW yellow patch), *penelope* Staudinger, 1897 (same with reduced dennis), *aglaope* Felder, 1862 (FW band distal to cell), *melpomene* (red forewing band), and *burchelli* (red forewing band and yellow hindwing stripe) fairly closely accompany the corresponding variations of *erato* (Map I), with a few notable exceptions in and near hybridization zones.

Heliconius hermathena Hewitson, 1853. Very rare and local in northern central Amazon from Santarém (or perhaps Belém?), Maués, and Manicoré to the far west (São Gabriel, Rio Negro). At Faro, occurs principally as subspecies *vereatta* Stichel, 1912, almost identical in color-pattern to *melpomene melpomene*; transitions are known between the nominate and mimetic subspecies.

Heliconius erato (Linné, 1758) (Plate V, figs. 50-51; Plate VI, figs. 63-64; Map I). Entire Amazon Basin, common. Major subspecies *amazona* Staudinger, 1896 (dennis-ray), *amalfreda* Riffarth, 1900 (dennis only), *estrella* Bates, 1862 (dennis-ray with reduced forewing band), *reductimacula* Bryk, 1953 (condensed FW yellow patch), *venustus* Salvin, 1871 (same with reduced dennis), *emma* Riffarth, 1901 (FW band distal to cell), *hydara* Hewitson, 1867 (red forewing band), and *phyllis* (Fabricius, 1775) (red forewing band and yellow hindwing stripe) are represented with approximate ranges and blend areas in Map I.

Heliconius ricini (Linné, 1758) (Plate IV, fig. 31). Maranhão and Amapá westward to Roraima (Bôa Vista) and Rondônia.

Heliconius demeter Staudinger, 1896. Almost entire area of Hylaea (excludes southeastern and north-central Amazon), but extremely local; at times common where found. Subspecies *bouqueti* Nöldner, 1902 (dennis-ray, with males imitating *egeria*), *beebei* Turner, 1966 (dennis only), *eratosignis* Joicey and Talbot, 1925 (partly coagulated FW yellow band and clearer rays), and *demeter* (FW band mostly distal to cell) closely follow the variations of *erato* (see Map I).

Heliconius sara (Fabricius, 1793) *thamar* (Hübner, 1806) (Plate IV, fig. 33). Entire area, common in many habitats.

Heliconius leucadia Bates, 1862 (Plate VI, fig.

61). Entire area from Maranhão to Uaupés, Benjamin Constant and Acre, always local and very much less frequent than *sara*. The nominate subspecies, with a white HW border, predominates over *pseudorhea* Staudinger, 1896 only in some populations northwestward.

Heliconius antiochus (Linné, 1767) *alba* Riffarth, 1900. Entire area except extreme southeast, commoner at the borders of the Hylaea in Mato Grosso and in Roraima. Form *zobeide* Butler, 1869 is most frequent in the lower middle Amazon; *salvinii* Dewitz, 1877 may · be found in extreme northeastern Roraima.

APPENDIX III

Systematic Changes, and Remaining Uncertainties

The new systematic arrangement of the silvaniform *Heliconius* is presented in Part V of this series. A total of six species is recognized (*ismenius, silvana, numata, hecale, ethilla,* and *pardalinus*), two more than those recognized by Emsley (1965) and with *hecale* much expanded. The largest uncertainties that still remain in the revision of this extremely complicated mimetic group, other than the placement of certain little-known subspecies, are the relationships of *Heliconius numata* to the *H.n. aristiona* and *H.n. aulicus* complexes, of *H. silvana* to *H.s. ethra*, and of these two species to each other; and of the northern *H. hecale* group of subspecies to the *H.h. quitalena* complex of the Amazon Basin.

The following species are added by the present paper to Emsley's lists of 1963, 1964, and 1965, defining the tribe Heliconiini:

Philaethria wernickei (separated from *P. dido*).

Agraulis lucina (separated from *A. vanillae*).

Eueides lampeto (separated from *E. vibilia*).

Heliconius astraea (separated from *H. egeria*).

Heliconius besckei (separated from *H. melpomene*).

Heliconius heurippa (separated from *H. melpomene*).

Heliconius timareta (separated from *H. melpomene*).

Heliconius luciana (added, provisionally being maintained separate from *H. elevatus*).

Heliconius eleuchia (separated from *H. sapho*).

Heliconius congener (separated from *H. sapho*).

The two species *Heliconius hygiana* and *H. clysonymus* are recombined, the latter name taking precedence over the former.

A number of taxonomic uncertainties still exist in the tribe. We have seen no specimens of the Peruvian *Dione miraculosa* Hering, 1926; from its original description, it may be a good species, isolated in southwestern Peru on the Pacific slope of the Andes. *Eueides procula* Doubleday, 1848 and *E.p. edias* Hewitson, 1861, while morphologically distinguishable, are connected in western Venezuela by a graded series (*E.p. luminosus* Stichel, 1903) and are probably conspecific. The situation of the *Eueides lybia* complex, however, is less clear; *E. lybia lybia, E. l. olympia* (Fabricius, 1793) and *E. l. lybioides* Staudinger, 1876 are allopatric, not connected by graded series, and morphologically distinguishable. While we favor maintaining them together, they may prove to be not interfertile. *Heliconius hecuba*, with which we have very limited field experience, may be separable into two sympatric species, though apparent intergrades are known in collections; the extremes of variation between *hecuba* Hewitson, 1857 at one end and *cassandra* Felder, 1862 at the other end of a sympatric population are quite far apart in many ways. Finally, until *H. hecalesia* and *H. longarena* are found flying together, the considerable possibility that they may be conspecific (linked by *H. h. gynaesia*) cannot be eliminated.

EXPLANATION OF PLATES

Plate I

FIGURE 1. *Philaethria dido,* Rio de Janeiro, ventral surface of hindwing, twice life size. Black, red, and green.

FIGURE 2. *Philaethria wernickei,* Curitiba, Paraná, ventral surface of hindwing, twice life size. Black and green.

FIGURE 3. Upper left (upside down): *Agraulis vanillae maculosa,* Xapuri, Acre.

Upper right: *Agraulis vanillae catella,* Xapuri, Acre.

Lower: *Agraulis lucina,* Alto Rio Juruá, Acre (identical to specimens from Xapuri).

All in the Museu Nacional, Rio. Dorsal, life size. Black and orange.

FIGURE 4. Six *Agraulis* from near La Merced, Junín, Peru, all ventral, life size. Orange, yellow, silver, and black.

Left row: three variations of *A. vanillae maculosa.*

Lower right: *A. vanillae catella* (note orange FW apex).

Upper and middle right: *A. lucina.* We also have specimens of *lucina* from this area with as much ventral silvering as the *catella* illustrated. All in the collection of G. Harris, Lima, Peru.

FIGURE 5. Upper left: *Eueides pavana,* male, Xerém, Rio de Janeiro.

Middle left: *Eueides pavana,* orange female, Petrópolis, Rio de Janeiro, 900 meters.

Lower left: *Eueides pavana,* intermediate female, Parque Nacional de Itatiaia, Rio de Janeiro (900 meters).

Upper center: *Eueides pavana,* yellow female, Belo Horizonte, Minas Gerais (1100 meters).

Middle center: *Eueides vibilia vibilia,* female, Conceição da Barra, Espírito Santo.

Lower center: *Actinote pyrrha* (Fabricius) (Acraeinae), male, Rio de Janeiro.

Upper right: *Eueides vibilia vibilia,* male, Conceição da Barra, Espírito Santo.

All dorsal, three-quarters life size. Black, yellow, and orange.

FIGURE 6. Types (upper male, lower female) of *Eueides nigrifulva* Kaye = *E. lampeto copiosus* Stichel, Potaro River, British Guyana, dorsal, one-half life size. Black and orange. From the Allyn Museum of Entomology.
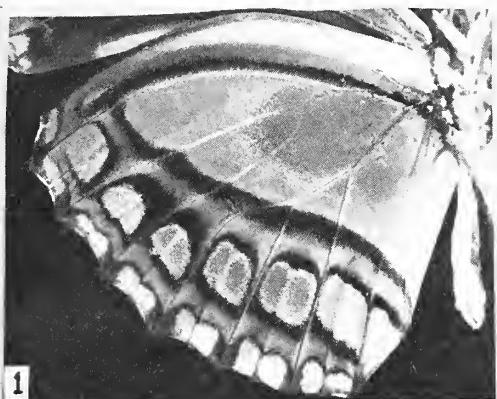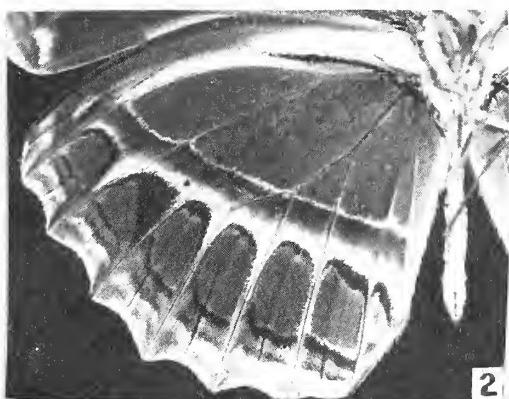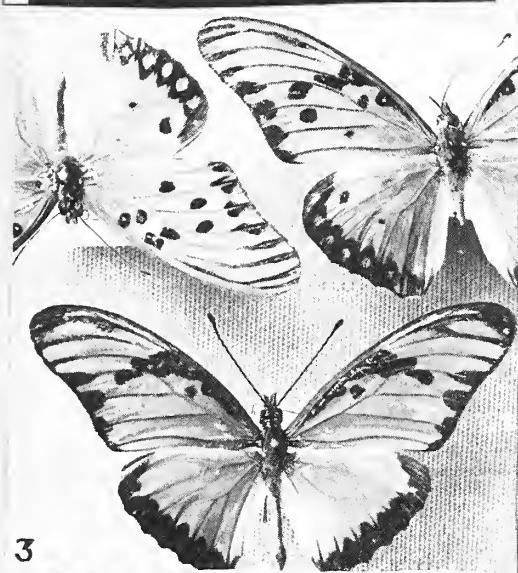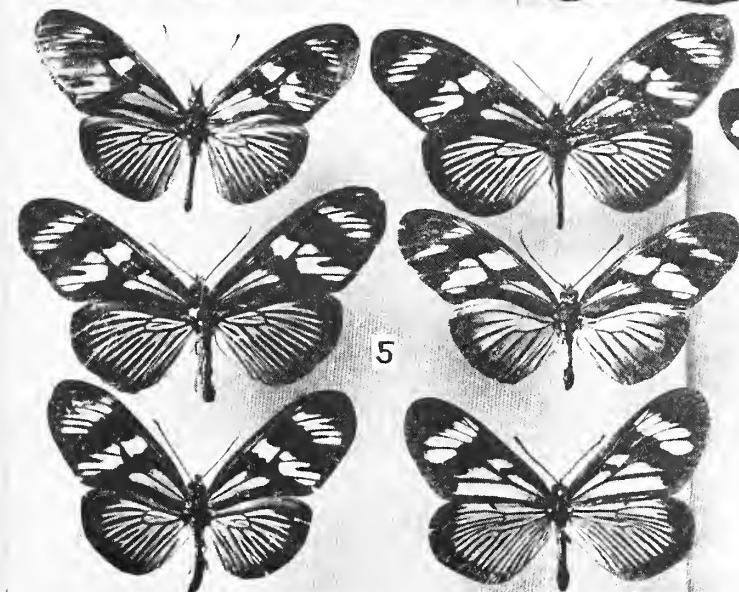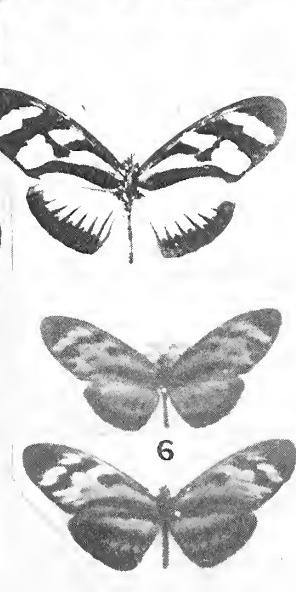
PLATE I

PLATE II

FIGURE 7. Upper left: *Heliconius congener* Weymer, 1890, Abitagua, Oriente, Ecuador (1100 meters). Iridescent blue and yellow.

Middle left: *Heliconius sapho sapho* (Drury, 1782), Victoria, Caldas, Colombia. Iridescent blue and white.

Lower left: *Heliconius eleuchia*(?) *eleusinus* Staudinger, 1885-8, highway from Medellin to Quibdó, northwestern Colombia. Black and white with reduced blue iridescence.

Upper right: *Heliconius eleuchia eleuchia* Hewitson, 1854, Victoria, Caldas, Colombia. Iridescent blue, yellow forewing bands, white hindwing border.

Middle right: *Heliconius eleuchia primularis* Butler, 1869, Santo Domingo, western Ecuador. Iridescent blue and yellow.

Lower right: *Heliconius hewitsoni* Staudinger, 1875, Agua Buena, Puntarenas, Costa Rica. Deep blue-black and yellow.

All dorsal, two-thirds life size.

FIGURE 8. Left: *Heliconius melpomene melpomene*, Rio Negro, Meta, Colombia, 900 meters. Black and red.

Right: *Heliconius heurippa* Hewitson, 1854, Rio Negro, Meta, Colombia, 900 meters. Black, red, and yellow. Both dorsal, two-thirds life size.

FIGURE 9. *Heliconius timareta* Hewitson, 1867, polymorphic population from the Rio Topo between Baños and Puyo, Ecuador, 1400 meters. Identical forms fly in the Abitagua, upper Santa Clara, and upper Rio Arajuno areas at 1000 to 1300 meters, and up the slopes of the valley of the Rio Pastaza to 1800 meters, in heavy humid forest.

Upper left: nominate form (black and yellow). Upper right: form *richardi* Riffarth, 1900 (black, yellow, and red).

Lower: two forewing band variants of form *contiguus* Weymer, 1890. Black, yellow, and red.

All dorsal, four-fifths life size. In the Carnegie Museum, Pittsburgh.

FIGURE 10. Intergradation of forms of *Heliconius melpomene* in eastern Ecuador. All forms may be found flying together on the northern escarpment of the Abitagua highlands, such as near Santa Clara (600-1000 meters), and Arajuno (500-1000 meters). The last two are found widely at higher elevations on the Rio Pastaza, such as at the Rio Topo, and elsewhere in eastern Ecuador at comparable levels. The first subspecies is widespread in the upper Amazon Basin of Brazil, Peru, Ecuador, and Colombia. The width of the blend zone near Santa Clara does not exceed twenty kilometers in horizontal and 500 meters in vertical dislocation.

Upper left: *H. melpomene aglaope* Felder, 1862, from near Arajuno. Black, yellow, and orange.

Upper center: form *adonides* Niepelt, 1908. Yellow forewing bands, orange dennis and rays.

Upper right: form *isolda* Niepelt, 1908. White forewing bands, red dennis and rays.

Lower left: form *niepelti* Riffarth, 1907. Red and white forewing bands, red dennis.

Lower center: *H. melpomene plesseni* Riffarth, 1907. Red and white forewing bands.

Lower right: form *pura* Niepelt, 1907. White forewing bands.

All dorsal, two-thirds life size.
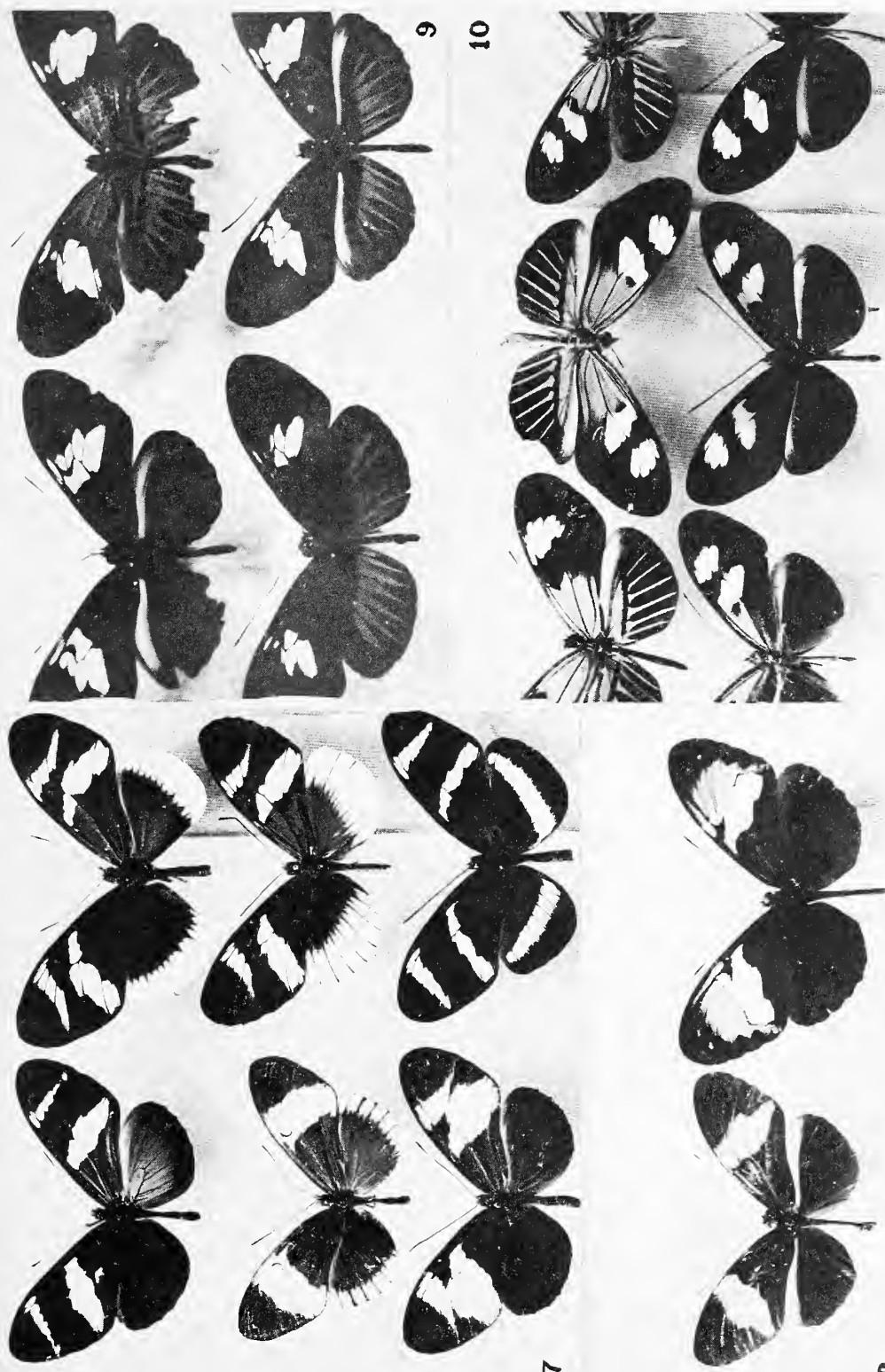
PLATE II

PLATE III

FIGURE 11. *Heliconius egeria* and *astraea* in Brazil.

Upper left and middle left: *H. astraea astraea,* São Paulo de Olivença, Amazonas; typical form.

Lower left: *H. astraea,* new subspecies (see Part IV), Manicoré, Rio Madeira, Amazonas.

Upper right: *Heliconius egeria hyas,* typical form, Maués, Amazonas.

Middle right: *Heliconius egeria asterope,* upper Rio Negro, Amazonas (typical *egeria* genitalia).

Lower right: *H. egeria egeria,* typical, Manicoré, Rio Madeira, Amazonas. Identical specimens are known from São Paulo de Olivença.

All dorsal, one-half life size. Black, yellow, and red. In the Museu Nacional, Rio de Janeiro.

FIGURE 12. Type-series of *Heliconius luciana,* extreme upper Orinoco River, Territorio Amazonas, Venezuela (2°11' N., 64°12' W.; Raudal "Los Tiestos").

Upper left: holotype male.

Upper right: allotype female.

Lower left: paratype female.

Lower right: paratype male.

All dorsal, two-thirds life size. Black and white. In the Facultad de Agronomia, Maracay, Venezuela (with permission of Dr. Francisco Fernández Yépez).

FIGURE 13. Six specimens of *Heliconius luciana* from the variable population at Mantecal, Rio Cuchivero, Bolivar, Venezuela. All dorsal, two-thirds life size. Black and yellow except for middle right specimen, which is black and white. Taken from a painting by the collector, H. Skinner of La Victoria, Venezuela, with his permission.

FIGURE 14. *Heliconius luciana,* male, Mantecal, Rio Cuchivero, Bolivar, Venezuela, dorsal, two-thirds life size. Black and yellow. In the Facultad de Agronomia, Maracay (donated by H. Skinner).

FIGURE 15. *Heliconius luciana,* male, Mantecal, Rio Cuchivero, Bolivar, Venezuela. Ventral, two-thirds life size. Black, yellow, and red.
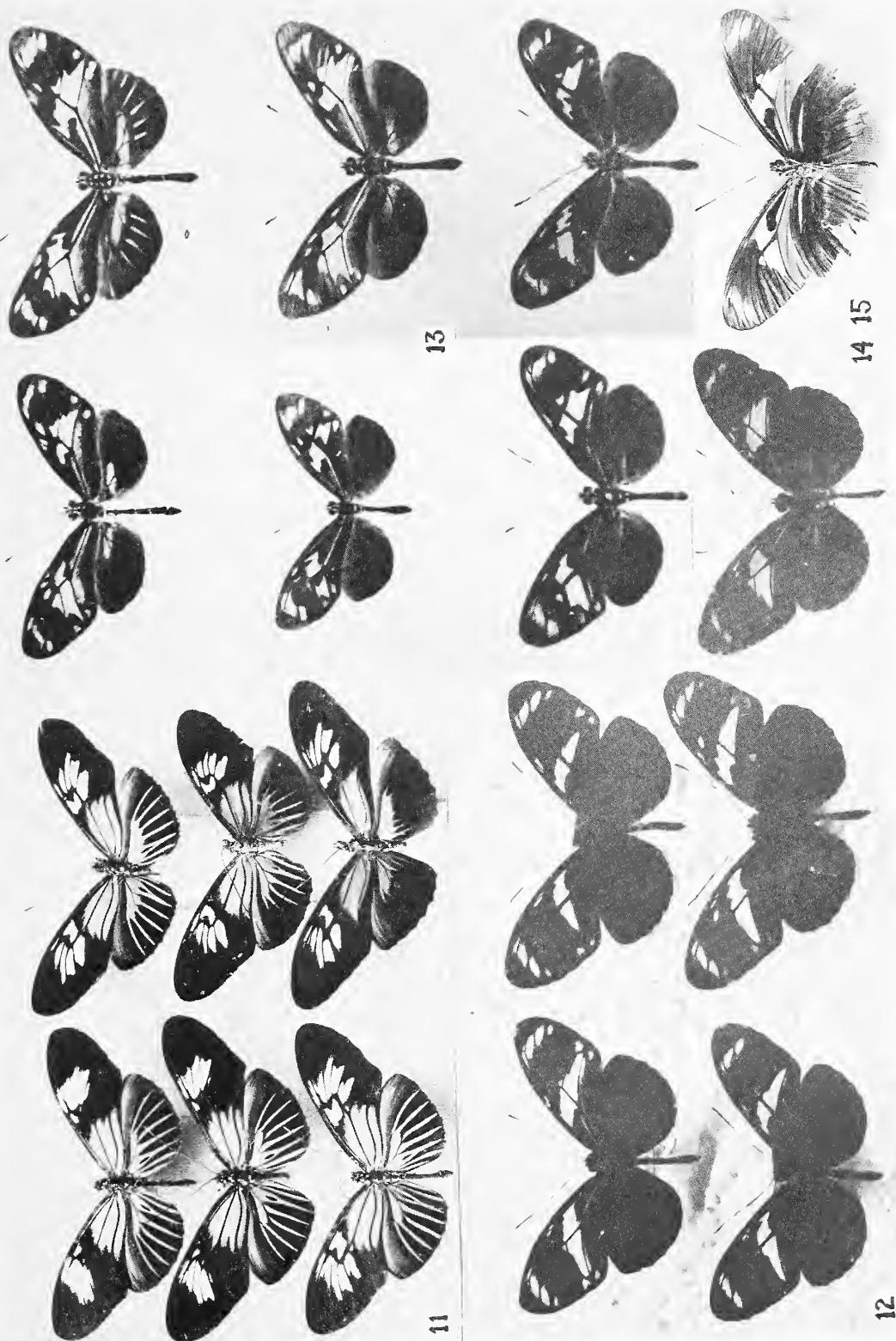
PLATE III

PLATE IV

Heliconians known from extra-Amazonian Brazil and not illustrated on other plates. Primitive genera, *Eueides*, primitive *Heliconius*, and most advanced *Heliconius* (*sara*-group).

Fig. 16: Black and green.

Figs. 17-24, 26: Black and orange.

Fig. 25: Black, orange, and yellow.

Fig. 27: Black, orange, and dull yellow.

Fig. 28: Black and orange.

Figs. 29, 32, and 33: Iridescent blue and yellow.

Figs. 30 and 31: Black, yellow, and red.

All dorsal, two-thirds life size.

FIGURE 16. *Philaethria wernickei wernickei,* Rio de Janeiro.

FIGURE 17. *Agraulis vanillae maculosa,* male, Itanhem, Bahia.

FIGURE 18. *A. vanillae maculosa,* female, Paraopeba, Minas Gerais.

FIGURE 19. *Dione juno juno,* male, Xerém, Rio de Janeiro.

FIGURE 20. *Dryadula phaetusa,* male, Rio Maranhão, Distrito Federal.

FIGURE 21. *D. phaetusa,* female, Barbacena, Minas Gerais.

FIGURE 22. *Dione moneta moneta,* male, Rio Claro, São Paulo.

FIGURE 23. *Dryas iulia iulia,* large male, Canal São Simão, Goiás.

FIGURE 24. *D. iulia iulia,* small female, Paraopeba, Minas Gerais.

FIGURE 25. *Eueides isabella dianasa,* male, Paracatú, Minas Gerais.

FIGURE 26. *Eueides vibilia unifasciatus,* male, Alto Garças, Mato Grosso.

FIGURE 27. *E. vibilia unifasciatus,* female, Alto Garças.

FIGURE 28. *Eueides aliphera,* male, Conceição da Barra, Espírito Santo.

FIGURE 29. *Heliconius wallacei flavescens,* male, Chapada de Guimarães, Mato Grosso.

FIGURE 30. *Heliconius xanthocles melete,* male, São Vicente, 90 km. E. of Cuiabá, Mato Grosso.

FIGURE 31. *Heliconius ricini,* male, Dom Pedro, Maranhão.

FIGURE 32. *Heliconius sara apseudes,* male, Belo Horizonte, Minas Gerais.

FIGURE 33. *Heliconius sara thamar,* male, Brasília, Distrito Federal.

PLATE IV
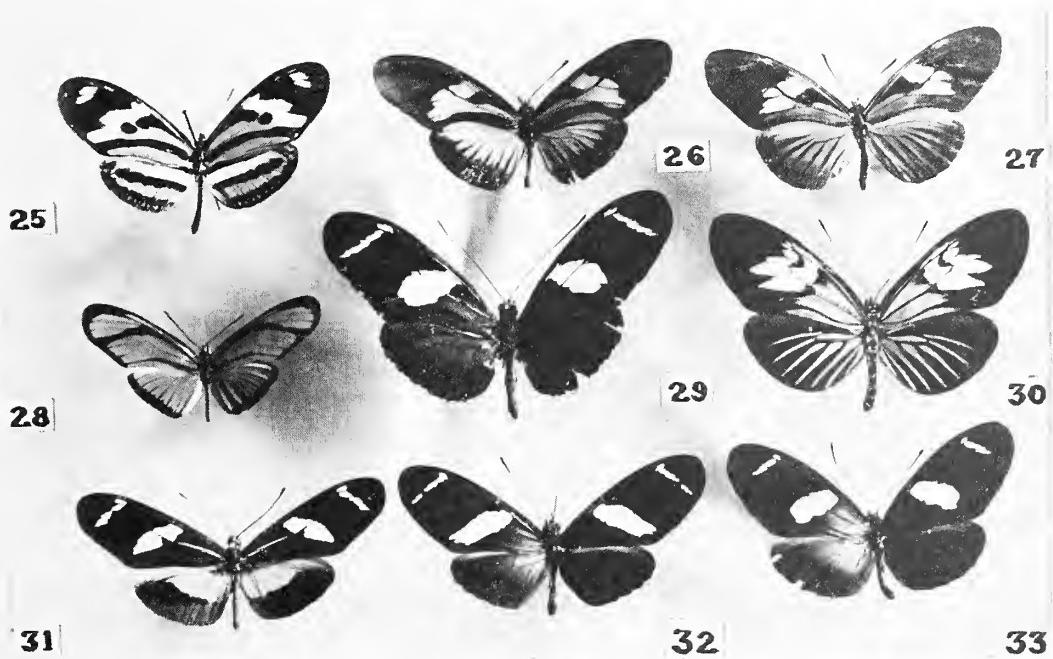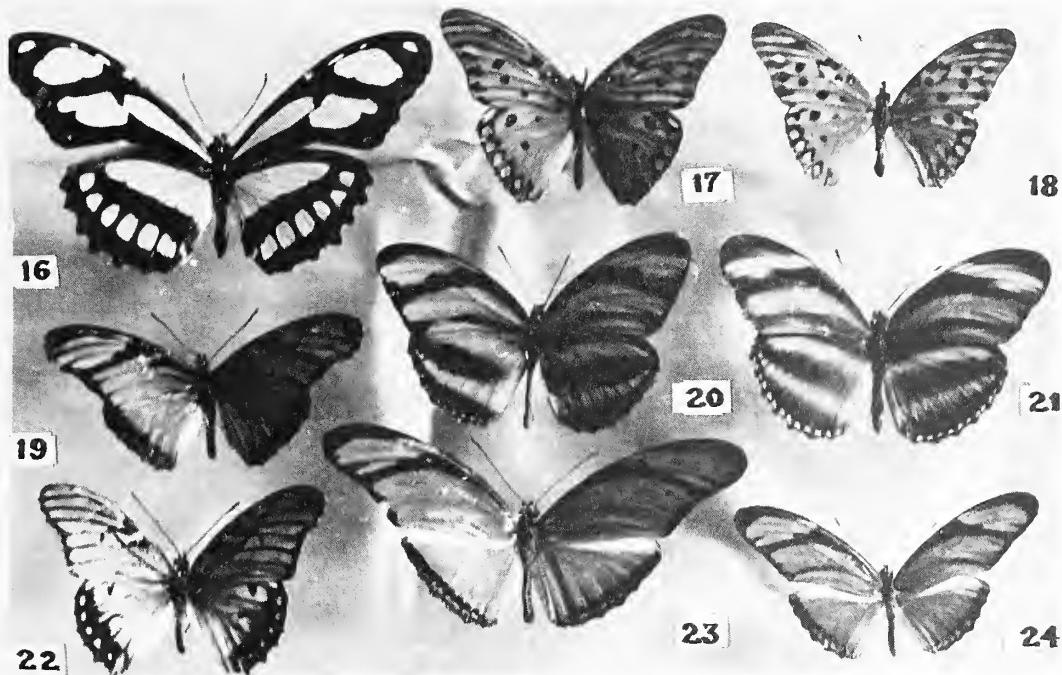
PLATE V

Heliconians known from extra-Amazonian Brazil and not illustrated on other plates (continued). Genus *Heliconius*: *silvana, melpomene,* and *erato* groups.

FIGS. 34-46: Black, yellow, and orange, with white subapical spot on forewing in 40-43, 45, and 46.

Figs. 47-51: Black, yellow, and red.
All dorsal, two-thirds life size.

FIGURE 34. *Heliconius silvana ethra,* male, Conceição da Barra, Espírito Santo.

FIGURE 35. *H. silvana ethra,* form *brasiliensis,* male, Recife, Pernambuco.

FIGURE 36. *H. silvana ethra,* variant, male, Conceição da Barra.

FIGURE 37. *H. silvana ethra,* form *brasiliensis,* variant, female, Conceição da Barra.

FIGURE 38. *H. silvana robigus,* male, Rio de Janeiro.

FIGURE 39. *H. ethilla flavomaculatus,* female, Recife, Pernambuco.

FIGURE 40. *H. ethilla narcaea,* light male, Rio de Janeiro.

FIGURE 41. *H. ethilla narcaea,* dark male, Santa Teresa, Espírito Santo.

FIGURE 42. *H. ethilla narcaea,* female, Santa Teresa.

FIGURE 43. *H. ethilla narcaea,* form *polychrous,* male, Loreto, São Paulo.

FIGURE 44. *H. ethilla eucoma,* male, Ubajara, Ceará.

FIGURE 45. *H. ethilla narcaea,* form *satis,* male, Rio de Janeiro.

FIGURE 46. *H. ethilla narcaea,* form *polychrous,* female, Loreto, São Paulo.

FIGURE 47. *Heliconius besckei,* male, Brasília, Distrito Federal.

FIGURE 48. *H. melpomene nanna,* male, Santa Teresa, Espírito Santo.

FIGURE 49. *H. melpomene burchelli,* male, Rio Maranhão, Distrito Federal.

FIGURE 50. *H. erato phyllis,* male, Rio de Janeiro.

FIGURE 51. *H. erato phyllis,* form *artifex* Stichel, 1899, male, Rio de Janeiro.
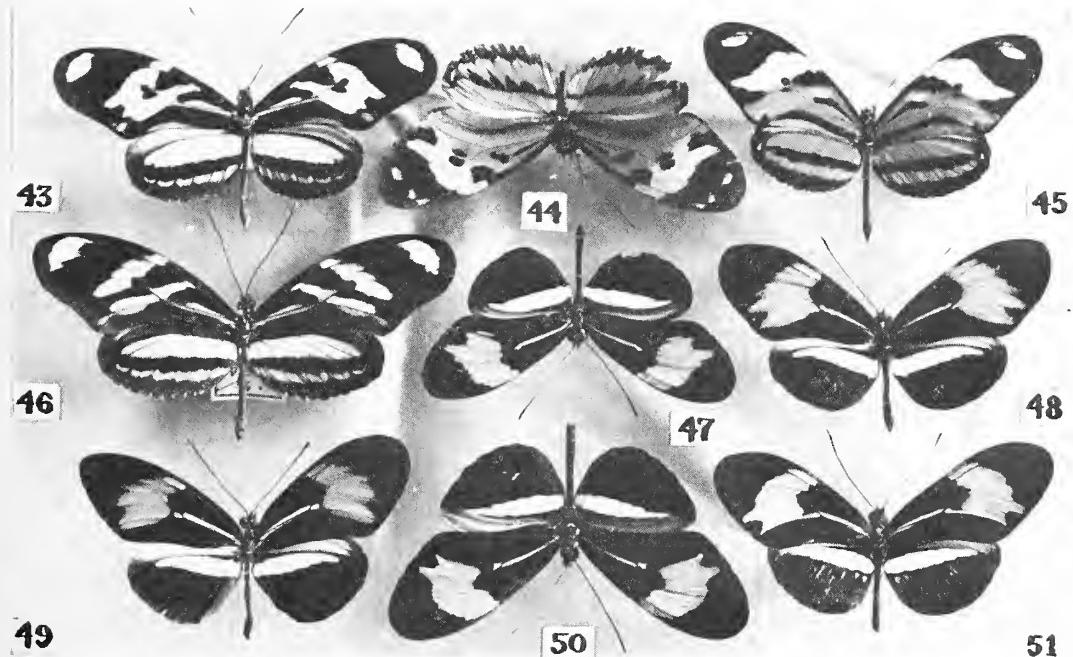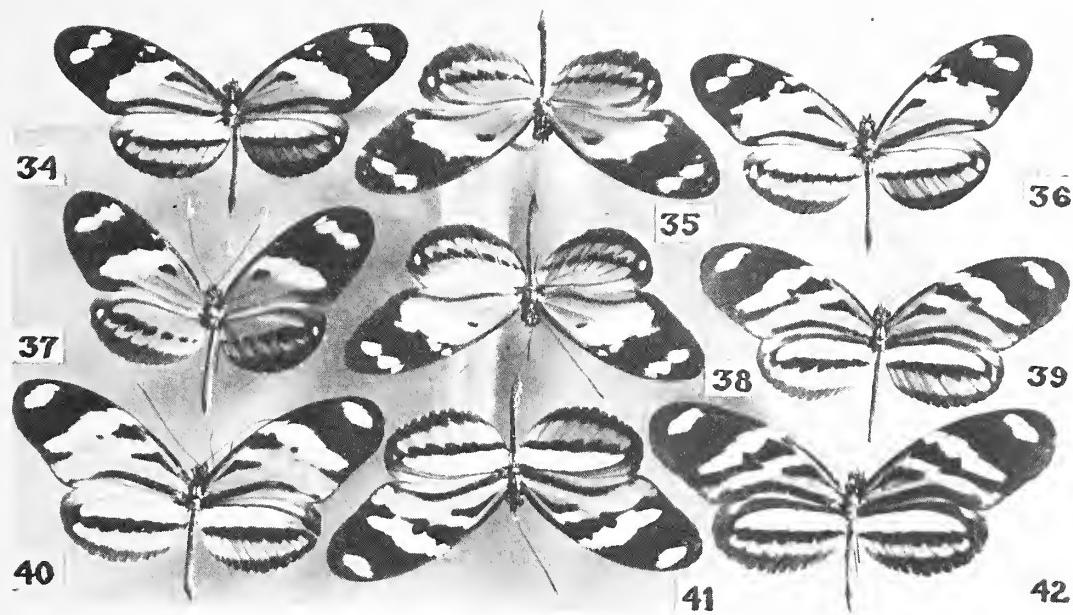
PLATE V

PLATE VI

Heliconians marginal in extra-Amazonian Brazil, not illustrated on other plates; polymorphism in Amazonian heliconians.

Figs. 52-60, 63, 64: Black, yellow, and orange to red.

Figs. 61 and 62: Iridescent blue and yellow.

All dorsal, two-thirds life size.

FIGURES 52-55. *Eueides isabella isabella,* variants from a single polymorphic population, all taken during 90 minutes' collecting on June 2, 1971, over a ten-meter radius in São Vicente, 90 Km. E. of Cuiabá, Mato Grosso, 600 meters. The total sample is 21 specimens.

FIGURES 56-58. *Heliconius numata* near *superioris* (upper male, middle and lower females), three variants from a fairly stable population, June 7, 1971, 17 Km. N. of Salto do Céu, upper Rio Branco (tributary of the Rio Cabaçal), west-central Mato Grosso, 400 meters.

FIGURE 59. *Heliconius silvana mirus,* near typical, male, Salto do Céu, Mato Grosso, June 7, 1971.

FIGURE 60. *Heliconius burneyi* near typical *burneyi,* male, Pimenta Bueno, Rondônia; identical to specimen observed near Salto do Céu, Mato Grosso, 400 meters, June 7, 1971.

FIGURE 61. *Heliconius leucadia pseudorhea,* male, Patrimônio Nôvo, upper Rio Jaurú, west-central Mato Grosso, 600 meters, June 10, 1971.

FIGURE 62. Variation in the population of *Heliconius wallacei* flying just north of Obidos, Pará. The second form from the top, plus combinants with a wider band, represents the bulk of the population. All taken in July 1970. Iridescent blue and yellow. Forms with white instead of yellow forewing bands, with all the illustrated band shapes, form up to one-quarter of the populations of *wallacei* in the northern middle Amazon; they may be still more abund-

ant northward, in areas where the white-banded *Heliconius antiochus* is the predominant species of the genus. This polymorphic population of *wallacei* may be found as far southwestward as the Manaus area.

FIGURE 63. Polymorphic hybrid population of *Heliconius erato* from Riozinho, 28 Km. down the Rio Machado from Pimenta Bueno, Mato Grosso, all taken in August 1970. The subspecies which meet here are *amazona* (upper left) from the northeast and *venustus* (lower right) from the south. A little farther downstream, *emma* also joins the gene pool from the west, producing a continuous series of polymorphic populations all the way down the Rio Madeira to Manaus, up the south bank of the Rio Negro to Barcelos, and westward to São Paulo de Olivença (see map).

Six principal forms can be recognized, for scoring members of populations along the Cuiabá-Pôrto Velho highway between Vilhena and the town of Rondônia, as follows:

(1) *amazona* Staudinger, 1896. Very open yellow band, full orange dennis and rays.

(2) (hybrid). Very open yellow band, dennis restricted to three lines and much redder.

(3) form *constricta* Joicey and Kaye, 1917. Yellow band closed down but still encircling much black, dennis orange and complete.

(4) (hybrid). Same, with dennis red and reduced.

(5) form *donatia* Fruhstorfer, 1910. Forewing yellow band almost totally compacted but still enclosing a small black spot or bar at or extending out from the end of the cell; dennis usually red and reduced.

(6) *venustus* Salvin, 1871. Forewing yellow patch compact, without black in center, and somewhat reduced distally; dennis red and reduced to three lines.

The eight specimens in Fig. 63 would be scored 1 — 2 — 1 — 4 — 4 — 3/5 — 5 — 6.

Analysis of some populations:

Km.	Elev.(m.)	Locality	form						total sample
			1	2 ·	3	4	5	6	
−200	700	South and west of Vilhena	—	—	—	—	3	12	15
0	600	Vilhena, frontier MT/RO	—	—	—	—	7	12	19
70	400	Km. 70, Vilhena-Pimenta Bueno	—	—	—	1	3	4	8
81	350	Km. 81, Vilhena-Pimenta Bueno	—	—	—	3	25	19	47
190	320	Pimenta Bueno	1	3	—	3	2	1	10
220	300	Riozinho, 1970 season	3	4	8	5	6	1	27
		Riozinho, 1971 trips	16	12	5	8	9	3	53
240	290	Km. 48 east of P. Bueno	3	2	3	1	—	—	9
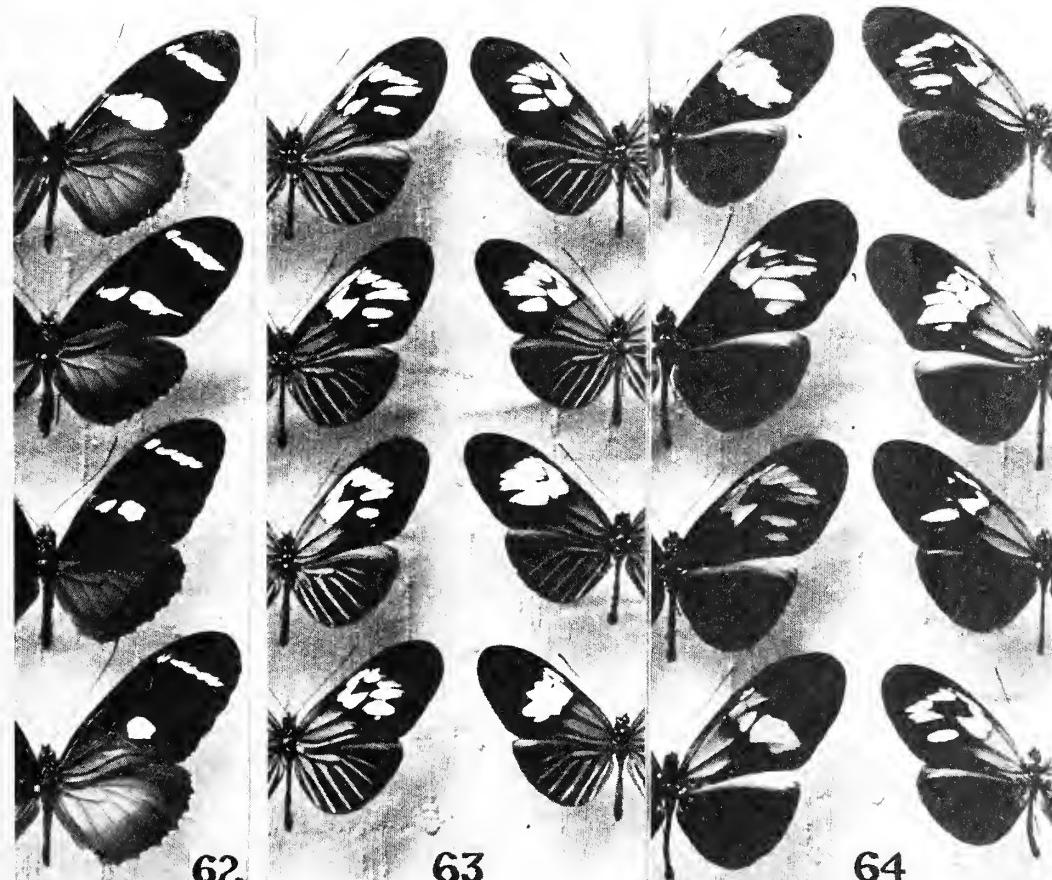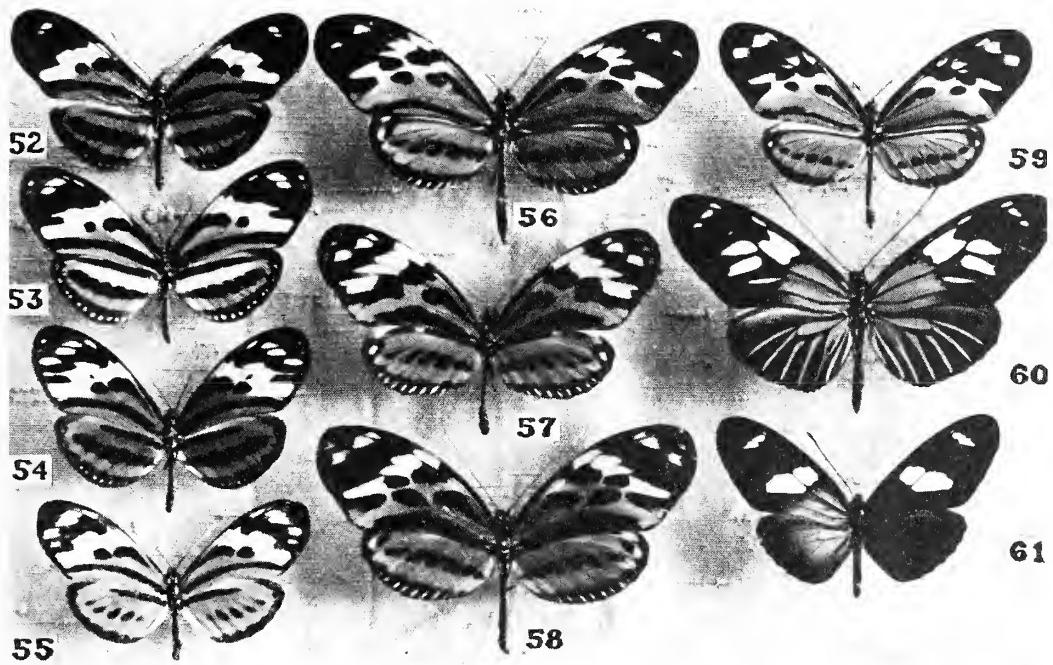
PLATE VI

FIGURE 64. Polymorphic hybrid population of *Heliconius erato* just north of Obidos, Pará. All taken in the same area in July 1970 except *cybelellus* (taken in the area in 1931). The approximate names of the forms are as below; the form *dryope*, with a *hydara* entire red band and dennis, is not illustrated; all forms except *hydara* and *amalfreda* may be considered as hybrid recombinants.

[intermediates here are known as *viculata* and *rubrizona*]	*hydara* Hewitson, 1867	*elimaea* (Erichson, 1848)
	belticopis Joicey and Kaye, 1917	*cybelellus* Joicey and Kaye, 1917
[intermediate, *callista*]	*callycopis* (Cramer, 1777) (Fig. F)	*helena* Riffarth, 1907
[typical form has a more irregular FW band than this specimen]	*coralii* Butler, 1877	*amalfreda* Riffarth, 1900
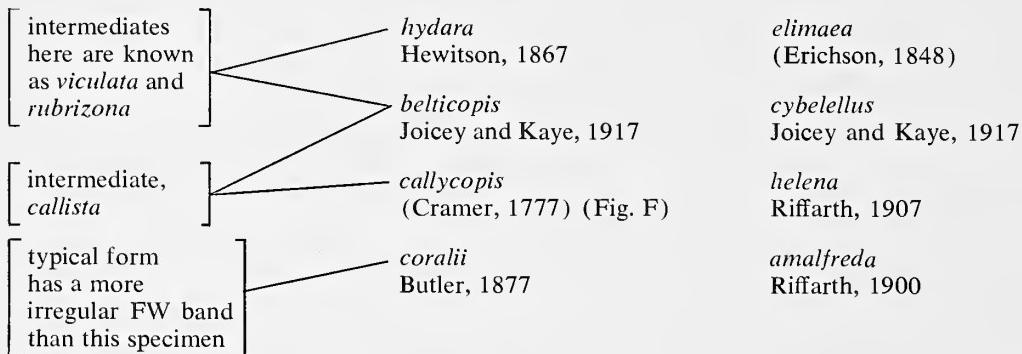
The approximate abundance of the forms in the 1970 population is (starting with the most common; total sample about 150 specimens): *amalfreda* — *hydara, viculata, rubrizona* — *helena* and varieties — *belticopis, elimaea,* and *coralii* — *dryope* — *callycopis* — *cybelellus.*

In the Obidos area, *Heliconius melpomene* is abundant and practically monomorphic as *m. melpomene,* with a black ground color and a broad red band on the forewing; a very few individuals have been taken with hybrid characters (dennis, or a mixed red-and-yellow forewing band). The first five illustrated forms of *erato,* and *e. dryope,* are very similar in flight to *m. melpomene,* appearing black with two bright red areas. The last three forms illustrated closely resemble the common sympatric *H. burneyi catherinae* and *H. tales.* Like these latter species, they fly higher, and are encountered more away from the streams, than the red-banded *erato* forms which fly lower and more slowly, with *melpomene* in the areas near permanent water. This double Müllerian mimicry in both behavior and pattern has apparently helped to stabilize an extremely large hybridization zone between *erato hydara* and *e. amalfreda,* covering almost the entire northern half of the lower middle Amazon (see map). This hybrid zone also extends south across the river to Santarém and Maués, where *hydara,* which is apparently able to cross the river, meets not with *amalfreda* but with the rayed *amazona;* these latter two forms, as well as dennis-rayed subspecies of *aoede, melpomene,* and *demeter,* seem to find an impenetrable barrier in the Amazon/Negro Rivers between the Ilha de Marajó and Barcelos. The stronger-flying species (*Eueides tales* and *Heliconius burneyi* and *egeria*) cross the river occasionally (like *erato hydara* and *m. melpomene*), producing populations polymorphic for rays in these species on both sides.

In Itacoatiara, on the north bank of the Amazon 200 Km downstream from Manaus, the hybrid population of *erato* is near its western limit. A sample of 13 specimens caught and another 15 seen indicates almost equal abundance of all of the forms illustrated, plus *dryope.* This suggests that this population may be composed principally of individuals of hybrid parentage, rather than the backcrosses of rarer hybrids to the parent subspecies as in Obidos. In Itacoatiara, the *m. melpomene* population shows greater signs of hybridization than in Obidos, but no yellow-banded individuals were seen or taken in a total sample of over 50. Many specimens, however, had signs of white or yellow in the underside, and one in the upperside, of the forewing red band. About one-quarter of the individuals also showed a dentate red line across the postdiscal area of the hindwing, possibly caused by a gene related to that which transforms *melpomene* rays from triangular to nail-shaped.

In the Manaus area, *erato* is monomorphic as *e. amalfreda.* Across the Rio Negro, only three Km away, the western dennis-rayed population with a polymorphic forewing band is found; there seems to be no gene exchange across the Rio Negro here.

2

The Heliconians of Brazil (Lepidoptera: Nymphalidae). Part III. Ecology and Biology of *Heliconius nattereri*, a Key Primitive Species Near Extinction, and Comments on the Evolutionary Development of *Heliconius* and *Eueides*

(Plates I-IV; Text-figures 1-4)

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The nearly unknown primitive butterfly species *Heliconius nattereri*, purported to be sexually dimorphic and seen but three times in this century, was relocated in steep humid primary forest near Santa Teresa, Espírito Santo, Brazil. A large colony was encountered in 1970, and a basic ecological and biological study was concluded during the late summer and fall expansion of the species (from mid-February to mid-May). The complete and accentuated sexual dimorphism was confirmed (the female was described and has been known as *H. fruhstorferi*). The juvenile and adult characters indicate the species to be very primitive in its genus, probably near the evolutionary line from *Dryas iulia* to the *silvana, melpomene, erato,* and *charitonia* groups of *Heliconius*. The egg, pupa, and adult morphology are very near those of the silvaniforms, while the larval stages show relationships to more primitive and more advanced heliconian species.

The adult males of *nattereri* promenade rapidly in strong sunlight on fixed paths in the upper levels of the forest, while the mimetic females are retiring and may rarely be observed on flowers, especially *Lantana camara* and *Gurania*.

The specialized adult behavior and restriction to a rare food-plant also used by other common, adaptable, and aggressive members of the genus are evidently the factors responsible for the apparent decline of *nattereri* since its discovery in the last century; its probable extinction is being greatly accelerated by man's destruction of its virgin habitat, with the creation of grossly disturbed areas in which *nattereri*'s more flexible relatives thrive.

Any scheme for the radial evolution of the genera *Heliconius* and *Eueides* must take into account the apparent relationships of *nattereri*, which show very little connection to *Eueides* or the *hierax, aoede, wallacei, xanthocles,* or *doris* groups of *Heliconius*. A very ancient bifurcation in the evolution line in the latter genus would place the above-mentioned groups in a "primitive" branch, and *nattereri* with the *silvana, erato, melpomene, charitonia, sara,* and *sapho* groups in an "advanced" branch. This evolution can then be graphed in such a fashion as to suggest the simultaneous appearance of certain characteristic mimetic color-patterns in members of diverse groups in the two genera. Most of the evolution of the group probably took place in the mid-Tertiary, especially the Miocene, though many subspecies were certainly formed during the Pleistocene. Some excellent examples of convergent, divergent, and parallel evolution are evident in the 55 species recognized in the two genera.

Two unusual red-banded species, *Heliconius hermathena* and *H. telesiphe*, are apparently little protected by Müllerian mimicry; they appear to be highly specialized to restricted biotopes (Amazonian sandy *cerrado* pockets and Andean pre-montane forest, respectively), and probably represent valuable evolutionary relics like *nattereri*, in need of intensive ecological and biological study.

Some generalizations on the distribution and behavior of relatively primitive and relatively advanced species of heliconians can be made, which cast some light on the evolution of the various subgroups of species. Much additional ecological fieldwork will be necessary in order to make these suggestions, as graphed and discussed, more firm and useful, in understanding tropical evolutionary trends on the larger scale.

Introduction

IN THE PREVIOUS PAPERS in this series (K. Brown, 1970; K. Brown and Mielke, 1972), we have discussed the history and recent rediscovery of *Heliconius nattereri*; described the tribe Heliconiini in Brazil; offered general comments on the species in the tribe; and presented a supplementary revision to the papers published by Emsley (1963, 1964, 1965). The detailed study of the near-extinct primitive species *H. nattereri*, discussed below, has indicated a fundamental reformulation of the evolutionary scheme advanced by Emsley (1965) for the genera *Heliconius* and *Eueides*. This reformulation is presented here as a descriptive graph, and discussed with relation to the 55 species recognized in this series of papers as belonging to the two genera.

ECOLOGY AND BIOLOGY OF *Heliconius nattereri*

At the time of Emsley's revision (1965), *H. nattereri* Felder and Felder, 1865, was the least-known of the heliconians, with the possible exception of *H. luciana*, omitted from that revision through an oversight (see Part II of this series). Emsley noted the existence of "less than eight specimens of each sex" of *nattereri* and indicated that the two sexes, very different in appearance, had not been captured together. The presumed female, *H. fruhstorferi* Riffarth, 1899, was placed in his revision as a simple color-form of *nattereri*, unassociated with sex.

At the beginning of this research program, we verified the existence of 13 males, all *nattereri*, and eight females, all *fruhstorferi*, in European collections, and none in American museums or the Allyn (ex Kaye) collection. At the beginning of our project, two additional males with accurate data were discovered in the Museu Nacional in Rio: one from Agua Preta, near Ilhéus, Bahia (= Fazenda São João, present-day town of Uruçuca, north of Itabuna and well inland from Ilhéus), September 1928, collected by E. May; and one from Santa Teresa, Espírito Santo, May 19, 1928, collected by E. Conde (from the Julius Arp collection). Eduardo May (1939) also mentioned seeing another male in the Uruçuca area in 1928, and observing a high-flying male near the Córrego Sabiá north of Colatina, Espírito Santo, in October 1936. No further specimens could be discovered in other Brazilian or Latin American collections, and no living collector could be found, even in Santa Teresa, who had seen the species alive in recent years.

In 1966, we instructed a resident insect collector in Santa Teresa, Claudionor Elias, to keep a lookout for *nattereri*. In the following year, he succeeded in collecting two males, on

March 15 and June 8, now deposited in the collection of the Departamento de Zoologia in Curitiba, Paraná. We then concentrated our work in the Santa Teresa area, and were able, over three years, to make a basic biological and ecological study of the species, and collect and breed several dozen specimens. Of these, we have placed three pairs and an additional female with unexpanded wings (mechanical difficulty during emergence from the pupa) in the Museu Nacional in Rio de Janeiro; one pair each in the Allyn collection (Sarasota, Florida), the Facultad de Agronomia (Universidad Central de Venezuela, Maracay), and the Carnegie Museum, Pittsburgh; one pair to O.H.H. Mielke (Curitiba, Paraná) and to Dr. J. R. G. Turner (York, England); and one male each in the collections of Harold Skinner (La Victoria, Venezuela), Francisco Romero R. (Maracay, Venezuela), Dr. E. W. Schmidt-Mumm (Bogotá, Colombia), Jorge Kesselring (João Pessôa, Paraíba), Gordon Small (Panama Canal Zone), and Dr. Helmuth Holzinger (Vienna, Austria). We are attempting to place the remaining specimens in all important *Heliconius* research collections around the world.* Ten males and three females were also collected by K. Ebert in February and March of 1970, and are in the H. Ebert collection in Rio Claro, São Paulo, and a further pair was taken in Santa Teresa by C. Callaghan of Rio in April 1971.

We have travelled in the territory presumably once occupied by *nattereri* in southern Bahia, northern Espírito Santo, and eastern Minas Gerais, but saw very little habitat suited to its demands (see below), and no further individuals of this species in areas other than Santa Teresa. Thus, all of the observations in this paper are drawn from field work in six colonies of *nattereri* discovered in the Santa Teresa area, at 500 meters to 900 meters elevation in dense primary Amazonian-type forest. A total of somewhat over 250 individuals has been observed during the three years of the study.

Heliconius nattereri is the only member of its genus to demonstrate strong sexual dimorphism (Plate I, figs. 1-6). The Guianian *H. demeter bouqueti* Nöldner, however, is moderately dimorphic in color-pattern and behavior, like *nattereri* (*fide* W. W. Benson; see Turner, 1966). The two sexes of *nattereri* occupy only poorly overlapping micro-habitats in the forest, and were evidently found together for the first time in our work in 1968. The number of specimens now known is sufficient to confirm a complete association of the color-forms with sex. This contrasts with the symmetrical distribution in both sexes of the two color-morphs of *H. ethilla*

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* If interested, please contact the author for details.

narcaea (*narcaea* and *satis*, discussed in Part II), sympatric with and quite similar to the female of *H. nattereri* in Santa Teresa. However, Turner (1968a) suggested that these morphs may eventually become wholly associated with sex, at least for the correspondingly dimorphic Trinidadian *H. ethilla ethilla*.

The black, yellow, and orange female of *nattereri* has a slow, casual flight except when startled, when it either mimics *Mechanitis* flight if mildly disturbed, or flies rapidly and directly upward and away if frightened. It joins very effectively the most common south Brazilian black-yellow-orange mimetic complex, whose principal distasteful members are silvaniform *Heliconius* (see Part V of this series) and itho-miines such as *Mechanitis lysimnia* and *polymnia*. A number of little-known ithomiine species from Bahia and Espírito Santo, notably *Hypothyris euclea laphria* and *H. daetina*, *Hyalyris fiammeta*, and *Napeogenes xanthone*, approximate in color-pattern the female of *nattereri* more than they do any other *Heliconius* species. A notable resemblance to the female of *nattereri*, even to identical red basal markings on the ventral hindwing surface, is achieved by the sympatric but much more widespread Batesian mimic, *Dismorphia astyocha* (Plate I, figs. 7 and 8). Even more similar in markings, but much smaller in size, is an unusual variant of *Phyciodes* (*Eresia*) *lansdorfi* very near to form *jacinthica* (Plate II, fig. 10); this form was

originally described, and today is principally known, from the *nattereri* faunal region. Two other Batesian mimics in this subgenus, *Phyciodes* (*Eresia*) *eunice esora* and the nearly unknown Bahian *P.* (*E.*) *erysice*, are also very similar in color-pattern to the female of *H. nattereri* (Plate II, fig. 10).

The females of *nattereri* apparently leave the deep forest to visit flowers a number of times daily—once in very early morning or on cloudy days at first clearing, and once or twice near midday (Table 1 and Graph 1), when they stay in the shadows and the undergrowth, approaching the flowers warily and leaving them quickly when satisfied. They seek out the food-plant (see below) to lay eggs in late morning and early afternoon, and may very rarely be seen otherwise, flying through the woods high above the ground or sunning on leaves near flowers or the food-plant (Table 1).

The yellow-and-black male of *nattereri*, when in high rapid flight through the forest, looks very much like a windblown dead leaf, due to a very fast, shallow, and irregular wingbeat. It also has been confused occasionally in life (by the author) with a number of smaller sympatric ithomiines (especially *Scada reckia*, *Napeogenes yanetta* and *sulphurina*, and *Aeria olena*), and with three day-flying Dysschematid (= Pericopid) moths, all of which have a much wider distribution than *nattereri* today (Plate II, fig. 11). The very rare and localized "splinter spe-

TABLE 1. FIELD BEHAVIOR OF *Heliconius nattereri*
(Total numbers of observations over four years)

| Hour (Day in Santa Teresa in March is 5:15 AM to 6:00 PM) | MALE | | | FEMALE | | | MALE COURTING FEMALE or other species |
	Visiting and feeding on flowers	Typical promenade	Sunning on leaves	Visiting and feeding on flowers	Inspecting and laying on *T. ovalis*	Other (sunning, flying)	
7:30– 8:00	1	–	–	5	–	1	–
8:00– 8:30	1	–	–	4	–	–	–
8:30– 9:00	11	–	–	6	–	1	–
9:00– 9:30	20	1	–	3	–	1	–
9:30–10:00	25	5	–	3	–	1	–
10:00–10:30	40	10	–	7	1	1	2
10:30–11:00	42	12	1	6	2	3	–
11:00–11:30	27	16	1	11	2	3	1
11:30–12:00	21	20	–	7	4	4	1
12:00–12:30	14	23	1	5	–	2	–
12:30– 1:00	15	33	1	8	4	3	–
1:00– 1:30	14	22	2	7	–	2	–
1:30– 2:00	5	16	3	1	–	1	–
2:00– 2:30	0	16	1	2	–	–	–
2:30– 3:00	1	9	2	–	–	1	–
3:00– 3:30	–	–	–	1	–	–	–
3:30– 4:00	–	–	1	–	–	–	–
4:00– 4:30	–	–	1	–	–	–	–
TOTALS	237	183	16/436	76	13	24/113	4

cies" of Pierid, *Perrhybris flava,* is known today only from the steep areas around Santa Teresa; the bright yellow male is very similar to *nattereri* males in flight, while the female, which we have not observed in nature, is very much like the female of *nattereri* in color-pattern (Plate I, fig. 9).

Males of *nattereri* visit flowers profusely in mid-morning (Table 1 and Graphs 1 and 2), and may pass over them or occasionally stop later in the day. Most of their time in the heat of the day is spent in promenade (Table 1 and Graph II), usually high above the ground on a set and repeatable path day after day, with the area covered estimated in four separate cases as over 50,000 square meters. The frequency for passing a fixed observation point in one direction is almost invariably fifteen minutes (in bright weather). The males always fly in the brilliant sun, and thus usually occupy the middle or upper story in dense forest; they rest on leaves during periods of cloud shade. In mid-afternoon, they may land with open wings on a sun-bathed leaf, usually high in a tree or vine (Table 1 and Graph 2).

Both sexes are observed most easily at flowers in the morning. The preferred flowers, when available, are the introduced but widespread red-and-yellow composite blooms of *Lantana camara* (Verbenaceae). Native red *Gurania sellowiana,* a cucurbitaceous vine also containing many flowerlets in a single head, is very frequently visited when in flower inside the woods. Where poinsettias (*Euphorbia pulcherrima*) of the less ornamental sort, with multiple yellow flowerlets surrounded by the red leaves, are introduced into the woods, they rapidly become the meeting and focal point for all local *Heliconius,* including *nattereri*; the heavily-visited blooms are produced from May through November. Other flowers occasionally visited by *nattereri* include small white orchids, magenta flowers of *Passiflora kermesina* (Plate I, fig. 6), blue and violet *Eupatorium* species, and red and yellow bromeliads. The males tend to visit or pass over flowers in the morning, before the high promenade period, at precise 15-minute intervals, suggesting that they may already be in a lower preliminary promenade, knowing that females also come to flowers during this period. Assuming from the breeding program (see below) a sex ratio near unity, the much larger number of observations of males over females on flowers attractive to both (Table
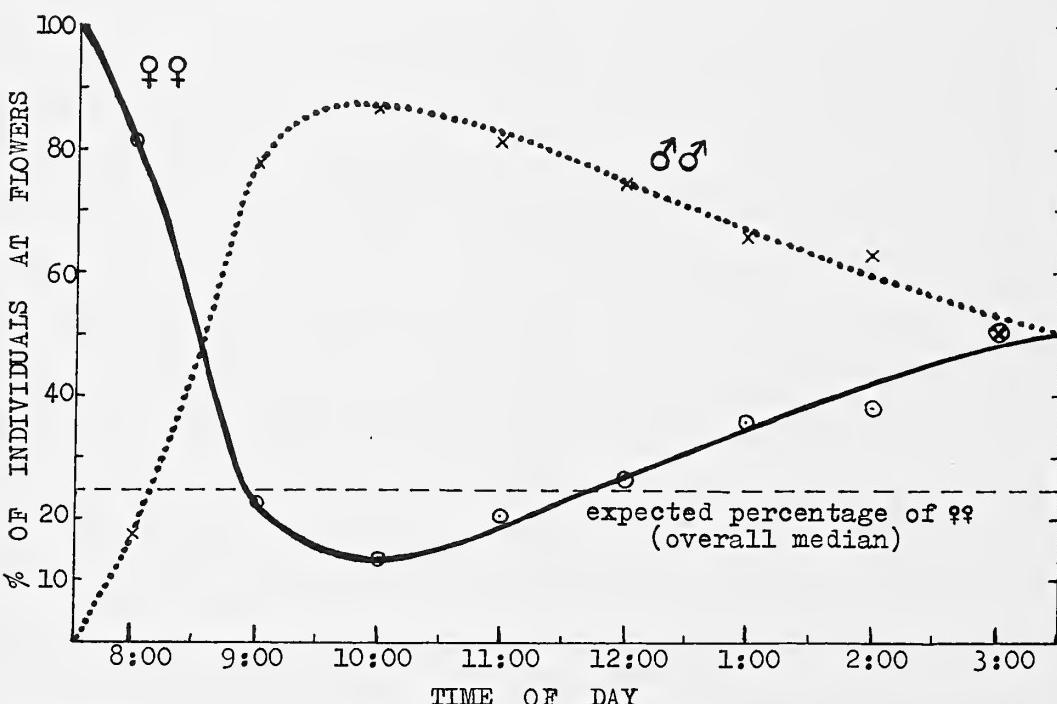
GRAPH 1. Flower-visiting of male and female *Heliconius nattereri,* plotted as percentage of each sex in the total individuals seen at flowers during each hour of the day's activity. Solid line: females; dotted line: males. Dashed line: median percentage of females in all the observations of *nattereri* at flowers. The females predominate in early morning, males in mid-morning. Data taken from Table 1.

1) may be due to the higher liquid requirements of the males, which promenade for hours in the midday sun, in relation to the females, which stay within the shaded woods and indeed have been observed almost exclusively during their furtive visits to forest-edge flowers.

The sexes are most likely to encounter each other in nature on or near preferred flowers in mid- to late morning; we have no evidence that virgin females produce any scent that could attract males from any distance. Males of any age will try to court young females, and also occasionally both sexes of the quite similar *H. ethilla narcaea*, when they notice them in the wild, but normally do not court in captivity. The aerial phase of the courtship is as seen in most heliconians (Crane, 1955, 1957), and the fanning by the male starts at the front or side, proceeds to the back, and returns to the front, over the stationary female with wings held half-open. Thus, the overall courtship is very similar to that of *Heliconius melpomene* (Crane, 1957).

In February to May 1970, *H. nattereri* was found to be truly common in one area on the edge of an enormous tract (over 100 Km²) of virgin forest east of Santa Teresa. This area (Table 2) included an exceptional abundance of flower food coupled with many food-plants in vigorous growth, in a setting of forests, clearings, and second growth which made observations and photography extremely easy. The exceptional population density present, with consequent frequent encounters between individuals, caused unusual quantitative (but not qualitative) changes in the species' normal behavior: males flew lower and more slowly, and promenaded near the ground over relatively smaller areas which were shared occasionally with other males, and both sexes visited low flowers, even in open areas, persistently and fearlessly. That the genetically determined behavior of butterflies is modified very often by environment and population density has been observed in a number of families and regions. The modification in this colony of the habits of *nattereri* to produce, from a wild, high-flying, inflexible, and nearly impossible-to-observe species, a heliconian much more like the common and adaptable species,
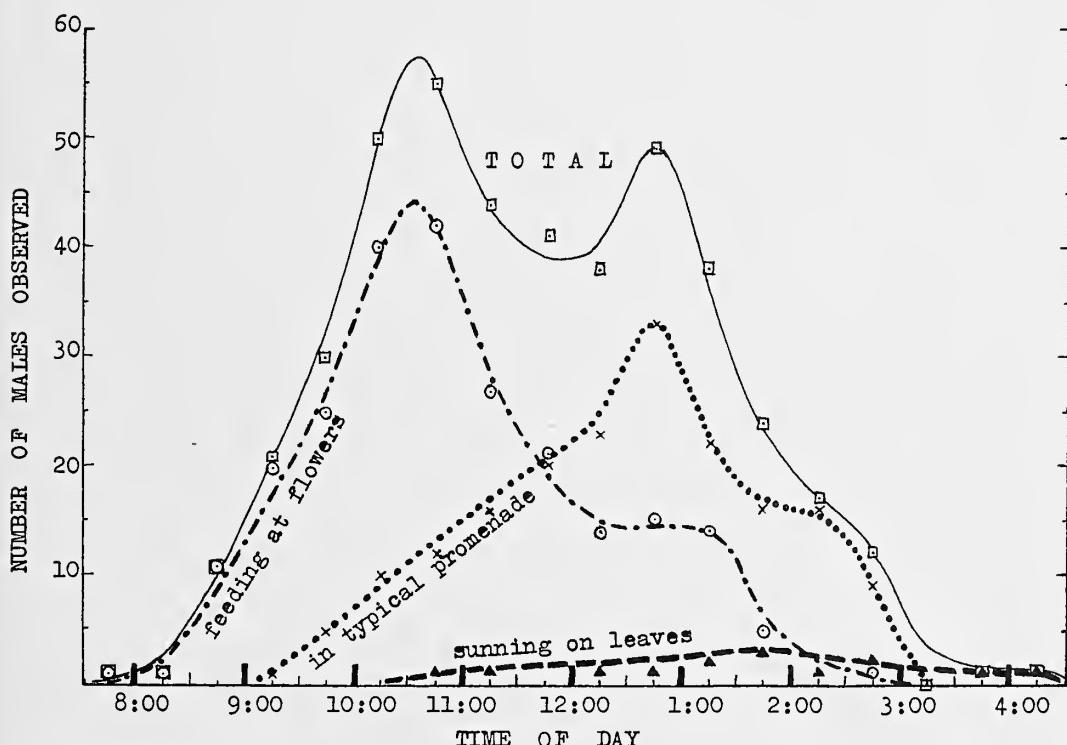
GRAPH 2. Distribution of the activities of male *nattereri* during the day, plotted as total number of sightings in each half-hour period (data from Table 1). Upper (solid) line: total sightings in all activities; the dip around midday may be real and is definitely *not* connected with decreased activity on the part of observers. Dotted and dashed line: flower visiting (peak in mid-morning). Dotted line: typical high promenading (peak in early afternoon). Dashed line: resting and sunning on leaves (peak in mid-afternoon).

TABLE 2. OBSERVATIONS OF *Heliconius nattereri* IN LARGE COLONY (MALE/FEMALE)

DATE (1970)	Effective man-hours observation	A	B	C	D	E	F	G	H	J	K	L	M	N	TOTAL SEEN*	7 MAN-HR. ADJUSTED TOTAL	RUNNING TOTAL*
February 27	6		-/1		4/1	1/-			1/-						6/2	9	6/2
March 1	2				1/-										1/-	4	6/2
March 4	8		1/-		1/2										2/2	4	7/3
March 11	5				3/2										3/2	7	10/5
March 12	6	-/1	-/1		1/1		1/-		2/-						3/2	6	12/7
March 16	5				-/2		1/-								1/3	6	13/10
March 21	5				1/1			1/-	1/1		1/-				3/2	7	16/12
March 22	7				1/2			4/-	1/2		4/-				8/4	12	23/15
March 23	6	-/1	-/1		1/-	1/-		2/-	-/1			-/1	1/-		7/3	12	30/17
March 25	7		1/-	1/-	3/-			2/-	1/-			2/2	2/1		11/4	15	39/19
March 26	10	-/1					-/1	3/-	1/-			3/-			6/2	6	42/20
March 27	1							2/-				2/-			2/-	14	43/20
March 28	12	-/1	1/-		3/2		1/-	4/-	-/1			2/-	3/1		14/5	11	54/24
March 29	12			1/-	1/-				1/-			3/2	1/-		6/2	5	58/26
March 30	12	-/1	-/1	2/-				-/1	1/-			1/1	1/-		5/4	5	62/29
April 3	5								1/2	1/1		2/1	2/-	1/-	7/4	15	67/32
April 4	6			1/-				1/-	1/1			2/-	2/-	1/1	8/2	12	71/33
April 5	7		2/-	2/-	1/1		1/-	-/1	1/-	1/-	1/-	3/1	1/-		12/3	15	79/36
April 6	2				1/1			1/-	1/-			2/2	1/-		6/3	31	81/38
April 10	1				2/1			1/-	1/-			2/1	1/-		3/1	28	83/38
April 12	1/2				-/1										-/1	28	83/38
April 13	4	1/1		1/-	1/2			3/-				1/-			6/3	16	89/41
April 17	4	1/1	1/-	3/-	1/2										3/3	11	92/43
April 18	6		1/1		1/-			3/-	1/-				1/-	-/1	10/2	14	101/45
April 19	3	-/1	1/-					1/-							3/1	9	102/46
April 21	3				2/1			2/-					2/-		6/1	16	105/47
May 2	6				6/3			1/2				1/-		1/-	6/3	11	110/50
May 4	4	-/1	-/1		4/3			4/2				1/-		1/-	4/3	12	113/52
May 11	4				2/1			-/2				1/-			4/3	12	117/54
May 13	1				1/-										1/-	7	117/54
TOTALS*	160-1/4	2/5	6/6	12/0	25/20	2/0	3/1	23/6	9/5	2/1	5/0	13/6	13/2	2/2	average adjusted total: 12		

* NOTES. The total observed each day represents separate distinguishable individuals.
The running total allows for duplication, by (a) collection of a part of those seen, and (b) recognition of the others by age, size, pattern marks, breaks, frays, etc.

EXPLANATION OF AREAS, SEPARATION AND CHARACTERISTICS:

The areas are roughly distributed along a straight line (with some lateral entrances), a fairly well-trodden path passing through forest, second growth, and cultivation.
The elevation of the area is 700-800 meters, the headwater basin of a small stream.

METERS FROM START	AREA	DESCRIPTION
0	A	Entrance to lower woods; much *Lantana*, visited by females in early AM.
110 + 90 lat	B	Clearing near creek where large tree felled in lower woods; *T. ovalis.*
180	C	Promenade area for males along trail and creek through lower woods.
270	D	*Lantana* patch where lower woods opens out by small creek; *T. ovalis.*
390	E	Trail crossing with one of two principal branches of stream, near top of lower woods; second growth, *Lantana.*
450	F	Exit from lower woods and second growth to fern field; woods edge.
550	G	Trail crossing of other principal branch of stream, in field; *Lantana.*
820	H	Entrance to upper woods, along a small creek; *Lantana*, woods edge.
960 + 70 lat	J	Small clearing in upper woods with much *T. ovalis.*
1000	K	Side exit of upper woods to cornfield; *Passiflora kermesina*, woods edge.
1090	L	Low *Lantana* flowers along edge of cornfield with second growth.
1170	M	Small *Lantana* bush along west edge of upper woods; woods edge nearby.
1390	N	Hilltop at end of upper woods, with clearing before final exit.

Communication between areas has been noted only for D-E and K-L-M.

like *erato, ethilla,* and *sara,* is an interesting witness to this phenomenon.

The evidently exclusive food-plant of *nattereri* in Santa Teresa was located in 1969; it is the little-known passifloraceous species *Tetrastylis ovalis.* This plant is apparently restricted today to the coastal area of Bahia and Espírito Santo (and possibly northern Rio de Janeiro) and may represent the limiting factor on *nattereri*'s present-day geographic distribution. It is an exceptionally slow-growing and high-climbing vine, which prefers deep virgin forest and produces extensive new growth (meristems) only at clearing edges or high in the forest canopy. As these limited growing tips are used for egg-laying not only by *nattereri,* but also by five widespread, common, and adaptable members of the same genus (*Heliconius silvana, ethilla, melpomene, erato,* and *sara*—the first four with intolerant and even cannibalistic larvae, the last with gregarious caterpillars which occupy a whole branch at a time), it is not strange that very few areas exist where substantial colonies of *nattereri* persist in the present day. It would seem to be near to its natural extinction, being displaced from the ecosystem by its more aggressive relatives, whose numbers have probably been increased by man's partial cutting of the forests (see below).

All attempts in Rio de Janeiro to breed adult *nattereri* from fertile eggs expressed from wild-caught females met with failure, as did efforts to induce these females to lay eggs on food-plant in a cage. In one case, however, a single larva, through the use of an air-conditioned room and constant care, was taken through the fourth stage before succumbing. The lack of fresh food-plant supply in the Rio area made essentially impossible a large-scale breeding program there. Therefore, eggs obtained from females captured in the above-mentioned large colony (up to four fertile eggs expressed from a single female over six days, though many females gave only one or none), and eggs and larvae found on food-plants discovered in the Santa Teresa area, were raised in March to May 1970 (Table 3) in pint jars kept in the shade in clearing B (Table 2). The jars were cleaned, and fresh leaves were provided twice a week. With this minimal care, and with the extensive handling of the larvae in photography, mortality in the later stages exceeded 50 percent, but enough adults finally were obtained (Table 3) to satisfactorily define the complete juvenile biology of the species, here illustrated and described.

EGG (Plate II, fig. 12): Bright yellow elongated ovoid truncated at bottom, 1.05 mm. to 1.20 mm. high, 0.75 mm. to 0.90 mm. in diam-

TABLE 3. BREEDING PROGRAM OF *Heliconius nattereri*

March 19 to May 18, in forest by food-plant (mean daily temperature probably about 18°–20° C.)

No.	SOURCE of juvenile	EGG			FIRST		SECOND		THIRD		FOURTH	
		laid	hatched	ds	molt	ds	molt	ds	molt	ds	molt	ds
1	Nature	19/III	24/III	5	28/III	4	31/III	3	2/IV	2	4/IV	2
2	Nature	21/III	27/LLL	6	30/III	3	2/LV	3	4/IV	2	7/IV	3
3	Nature	21/III	27/III	6	30/III	3	2/IV	3	4/IV	2	7/IV	3
4	Female— 1	22/III	28/III	6	31/III	3	3/IV	3	5/IV	2	8/IV	3
5	Nature	–	–	–	–	–	3/IV	–	5/IV	2	9/IV	4
6	Female— 1	23/III	29/III	6	1/IV	3	4/IV	3	6/IV	2	9/IV	3
7	Nature	23/III	30/III	7	2/IV	3	4/IV	2	6/IV	2	10/IV	3
8	Nature	23/III	30/III	7	2/IV	3	4/IV	2	6/IV	2	10/IV	3
9	Female— 1	25/III	30/III	5	2/IV	3	4/IV	2	6/IV	2	11/IV	5
10	Female— 2	25/III	2/IV	8	4/IV	2	7/IV	3	10/IV	3	13/IV	3
11	Female— 1	26/III	1/IV	6	4/IV	3	§	–	–	–	–	–
12	Female— 3	26/III	1/IV	6	4/IV	3	§	–	–	–	–	–
13	Female— 3	27/III	2/IV	6	4/IV	2	6/IV	2	9/IV	3	13/IV	4
14	Female— 4	28/III	3/IV	6	5/IV	2	8/IV	3	11/IV	3	14/IV	3
15	Female— 3	28/III	3/IV	6	6/IV	3	9/IV	3	11/IV	2	14/IV	3
16	Female— 5	28/III	3/IV	6	6/IV	3	8/IV	2	11/IV	3	14/IV	3
17	Female— 6	28/III	3/IV	6	6/IV	3	9/IV	3	11/IV	2	§§	–
18	Female— 4	29/III	4/IV	6	6/IV	2	9/IV	3	12/IV	3	§§	–
19	Female— 5	29/III	5/IV	7	7/IV	2	10/IV	3	13/IV	3	19/IV	6
20	Female— 7	30/III	5/IV	6	7/IV	2	10/IV	3	13/IV	3	17/IV	4
21	Female— 5	30/III	5/IV	6	7/IV	2	9/IV	2	13/IV	4	17/IV	4
22	Female— 8	30/III	5/IV	6	7/IV	2	10/IV	2	13/IV	3	17/IV	4
23	Female— 9	30/III	5/IV	6	7/IV	2	10/IV	3	13/IV	3	18/IV	5
24	Female— 7	3/IV	6/IV	3	9/IV	3	13/IV	4	16/IV	3	20/IV	4
25	Female— 8	3/IV	6/IV	3	§	–	–	–	–	–	–	–
26	Nature	–	–	–	–	–	–	–	17/IV	–	20/IV	3
27	Nature	6/IV	13/IV	7	16/IV	3	20/IV	4	24/IV	4	4/V	10
28	Female—10	12/IV	17/IV	5	20/IV	3	24/IV	4	28/IV	4	3/V	5
29	Female—11	13/IV	20/IV	7	24/IV	4	28/IV	4	3/V	5	6/V	3
30	Female—12	13/IV	20/IV	7	24/IV	4	28/IV	4	§	–	–	–
31	Nature	–	–	–	3/V	–	6/IV	3	9/V	3	14/V	5
32	Nature	–	–	–	3/V	–	6/IV	3	9/V	3	14/V	5
33	Nature	29/IV	4/V	5	7/V	3	10/V	3	13/V	3	16/V	3
34	Nature	30/IV	5/V	5	8/V	3	11/V	3	14/V	3	(x)	–
35	Female—13	2/V	9/V	7	13/V	4	15/V	2	17/V	2	(x)	–
36	Female—14	4/V	11/V	7	14/V	3	16/V	2	(x)	–	–	–

| average duration of stage: | | | | 6.0 days | | 2.8 days | | 2.9 days | | 2.8 days | | 4.0 days |
| mortality in stage (%) | | | | (0) | | 3 | | 6 | | 3 | | 7 |

† = stage shortened in Rio (25°)
§ = died during instar (or pupa)
§§ = died in molt
(x) = preserved at end of program

eter, with 14 (rarely 16) vertical and eight to nine regular horizontal ridges (plus two to three irregular ridges in hemispherical area at top); laid singly very near the tip of a tendril (rarely, a small leaf) on a vigorously growing meristem of *Tetrastylis ovalis* Vell.; duration normally five to seven days, exceptionally three to eight days (two to five days is normal in other *Heliconius*).

LARVA: First instar, uniform yellow and 2.0 mm. long upon hatching, does not normally eat its eggshell, perhaps a sign (Alexander, 1961a) of the tolerance it shows throughout all its larval life towards other larvae, even those much smaller than itself; two or rarely three larvae often peacefully occupy the same meristem. Although this disposition would be helpful in maintaining a colony in the presence of limited foodplant, it would be fatal in the presence of competition for this plant by non-tolerant larvae of the more adaptable and common *Heliconius* species (see discussion above).

Mature first instar (Plate II, figs. 13 and 14) dark yellow with brown legs, variable but usually weak development of dark pigment spots and/or bands, and a deep yellow-brown head

Fifth molt	ds	Pupa color	hatch	ds	Adult sex
11/IV	7	D	26/IV	15	M
14/IV	7	D	27/IV	13	M
14/IV	7	D	27/IV	13	F
15/IV	7	L	28/IV	13	F
15/IV	6	L	§	–	–
15/IV	6	D	§	–	–
17/IV	7	L	29/IV	12†	F
§	–	–	–	–	–
§§	–	–	–	–	–
21/IV	8	LD	7/V	16	F
–	–	–	–	–	–
21/IV	8	L	§	–	–
21/IV	7	D	7/V	16	F
22/IV	8	L	§	–	–
22/IV	8	L	7/V	15	M
–	–	–	–	–	–
26/IV	7	L	§	–	–
24/IV	7	L	§	–	–
§	–	–	–	–	–
§	–	–	–	–	–
25/IV	7	LD	13/V	18	F
27/IV	7	D	14/V	17	M
–	–	–	–	–	–
27/IV	7	D	14/V	17	F
§§	–	–	–	–	–
13/V	10	L	23/V	10†	M
§§	–	–	–	–	–
–	–	–	–	–	–
(x)	–	–	–	–	–
(x)	–	–	–	–	–
(x)	–	–	–	–	–
–	–	–	–	–	–
–	–	–	–	–	–
–	–	–	–	–	–
	7.3 days		14.6 days		
	25		33		

ringed posteriorly with dark brown (ring tapering dorsally and indenting cephalad to meet head suture); area around pseudoocelli dark brown; setae dark but semi-translucent (chaetotaxy to be published opportunely); maximum length 5.5 mm to 6.0 mm; normal duration three, exceptionally two or four, days.

Second instar (Plate II, figs. 15 and 16) dark orange-brown, with not highly visible pigment spots (in same pattern as in later instars) and an entirely black head; scoli and legs dark; head scoli 0.5 mm to 0.6 mm, head height 0.8 mm to 0.9 mm, dorsal thoracic and abdominal scoli up to 1.0 mm in length; thoracic scoli noticeably longer, and anal scoli much shorter, than abdominal scoli in this and later instars; maximum length 9 mm to 10 mm; duration normally three, occasionally two or four, days.

Third instar (Plate III, fig. 17) very similar, but with some individuals closer to fourth-instar pattern; in general slightly to much lighter than second instar, tending to progressively lighten dorsally during the instar, with dark pigment-spot pattern also clearly visible by the end; head either almost uniformly dark brownish, or somewhat lighter dorsally and darker ventrally, suggestive of fourth instar head; head, sublateral, and anal scoli about equal to head height (1.1 mm to 1.3 mm), dorsal and supralateral scoli up to 1.3 times head height; maximum length 14 mm to 16 mm; duration two, three, or four days.

Fourth instar (Plate III, figs. 18 and 19) dorsally and laterally green to whitish with well-developed large black pigment spots (distribution as in Text-fig. 1, size larger), ventrally (below sublateral scoli) dark brown, prolegs dark basally and yellow at the tips; head (Plate III, fig. 21) rounded, principally dark to bright yellow dorsally and laterally, dark brown to nearly black ventrally and frontally, with dark areas around the eyes and extending up the frontal sutures and bifurcating, inner forks following frontal sutures upward and inward but not meeting, wider outer branches extending to base of scoli; frontal plate black, sutures and ring around dark mandibular area yellow; prothoracic plate black, divided; all scoli dark; anal, sublateral, and recurved head scoli 2.5 mm to 2.7 mm, head height 1.8 mm to 1.9 mm, dorsal and supralateral scoli up to two times head height; maximum length 22 mm to 25 mm; duration normally four days, but occasionally three, five, or more days, depending upon food-plant supply.

Fifth instar (Plate III, fig. 20) lighter (light greenish to pure white) dorsally, pure white laterally, and deep brown ventrally (except for tips of prolegs); pigment spots smaller than in fourth instar (Text-fig. 1), but never obsolescent even in light individuals; head (Plate III, figs. 22 and 23) rounded, light to medium yellow; mandibles and adjacent area, region around the eyes, and center of frontal plate black; a black sickle-shaped mark initiating in front of each ring of ocelli (usually fused with dark ocular area), and curving forward, inward, and then upward to follow the frontal sutures, and then outward to meet the base of each scolus; prothoracic plate black; all scoli black, with head scoli 4.6 mm to 5.6 mm, head height 2.5 mm to 3.0 mm, and dorsal scoli up to three times head height; maximum length 33 mm to

36 mm; duration five to six days to maturity.

The mature larva, after last defecation, turns a creamy white color (retaining pigmented areas on head and body, however), and hangs in an inverted position for 24 to 30 hours (Plate III, fig. 24) in a suitable location, before transforming to the pupa.

The total duration of the larval stage is normally 18 to 21 days, in the climate of late summer in Santa Teresa (mean temperature slightly less than 20° C). This period, like those for the egg and pupa, is somewhat shortened if there is considerable incidence of direct sunlight, or if the breeding is carried out at higher temperatures (as in Rio de Janeiro, late summer mean about 25° C). The larval stage is normally 13 to 15 days in *Heliconius* (see Beebe, Crane, and Fleming, 1960).

CHRYSALIS (Plate III, figs. 25 to 30): hangs vertically without strong bows or flexions, but with wings projecting well below abdomen; nearly identical in form to that of *Heliconius melpomene* (Beebe, Crane, and Fleming, 1960), more slender than that of *H. ethilla narcaea*; total length 25 mm; color dark brown or light tan (occasionally intermediate), unrelated to sex, with six large strongly reflective patches on the dorsal surface of the metathorax and first

two abdominal segments, and four small reflective patches, two on the prothorax and two on the second abdominal segment; in dark pupae, six lighter streaks, and in light pupae, six dark streaks along the abdomen; two short broadly branched cephalic projections; about 27 short spines along each antenna case; six subequal medium-length spines in two rows on the dorsal side of the fifth to seventh abdominal segments; short spines on the anterior part of each large reflective area and on the strongly humped second thoracic segment; a pair of flanges (light or dark in color as the pupa) on the third and fourth abdominal segments, bearing on the third segment a long spine directed outward and forward, and on the fourth segment a relatively short spine.

When the pupa is disturbed, it makes rapid swinging motions through lateral bending at the fifth abdominal segment; these are unaccompanied by any noise or odor, and are frequently arrested at the end of the swing, leaving the pupa in a crooked position for a short period of time.

The duration of the pupa in Santa Teresa averages 15 days, unusually long for heliconians. The adult emerges (Plate IV, figs. 31 to 39) in very early morning (5 A.M. to 8 A.M.); the sex can usually be told by the appearance of
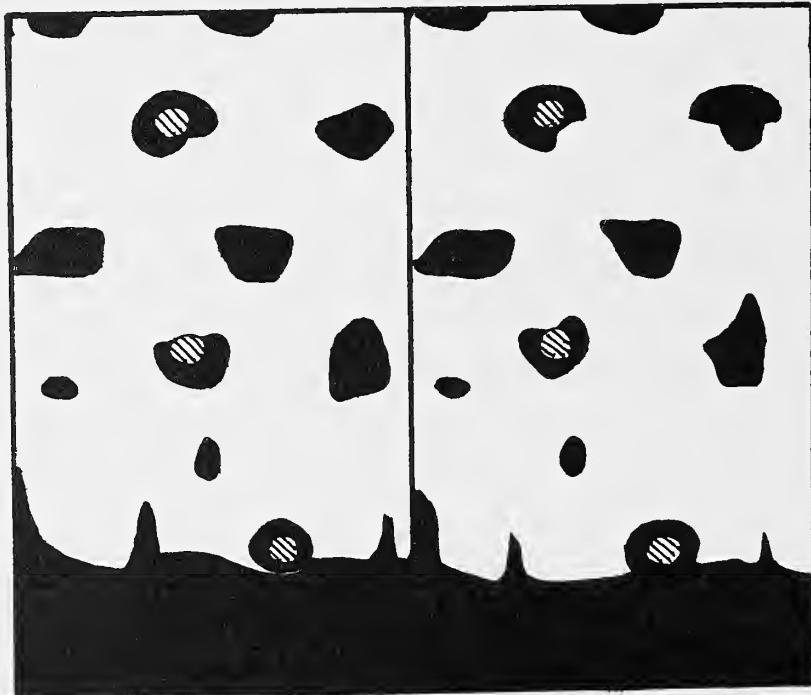
TEXT-FIGURE 1. *Heliconius nattereri,* mature larva, pigment patterns on third and fourth abdominal segments, from dorsal midline to base of prolegs, schematic.

the characteristic wing-pattern on the morning of the previous day. The wings are fully expanded within five minutes of emergence, and the first meconium, voided after ten to 15 minutes, is deep chestnut-colored, almost brown. First flight is before midday, though strong flight and feeding occur only on the second day (see Alexander, 1961b).

ADULT: We have noted very little variation and no aberration in the adults seen and studied. In the males, the dark area of the underside may be infused with silvery scaling (Plate IV, fig. 39), and the widths of the three yellow bands on the dorsal wing surface are somewhat variable; in a minority of cases, the cubital and postdiscal bands on the forewing converge at a point, breaking the dark band which separates them into two triangles. The overall effect of a flying male can be appreciably lighter or darker, depending upon this variation. Females have a variable width to the yellow postmedian band of the forewing and the median band of the hindwing; the latter is often darker yellow or orange like the submarginal stripe and the forewing cubital band. The two forewing bands very rarely extend to meet (Plate I, fig. 3), as occurs more frequently in males, and the cubital bar is sometimes suffused with yellow scaling

basally and/or distally. On the underside, the dark areas are frequently infused with reddish, rarely with silvery scaling.

The normal lifespan of adults in nature is probably over one month, and at least one recognizable male has been observed over a period of 11 weeks in one of the colonies near Santa Teresa.

The morphology of the male is near to that figured in Emsley (1965), except that the androconia seem restricted to forewing vein 1A and hindwing veins Sc + R_1 and Rs (not found by us in careful searching on other veins or on the membrane); the genital valves are typically silvaniform. We cannot confirm the female morphology reported by Emsley. The bursa copulatrix (Text-fig. 2) bears medium long, three- to four-toothed signa similar to those of *H. aoede* and *H. doris,* lacking only a right-angle bend instead of an arc at the lower end to be typically melpomeneform (see Part II of this series). The abdominal processes (Text-fig. 3) are strongly curved at the base, as in members of the *melpomene-group,* and the specialized scales borne by them (Text-fig. 4) are conical and three-pronged. The spermatheca has a diverticulum connected by a broad duct, as in almost all *Heliconius* species, and the paro-
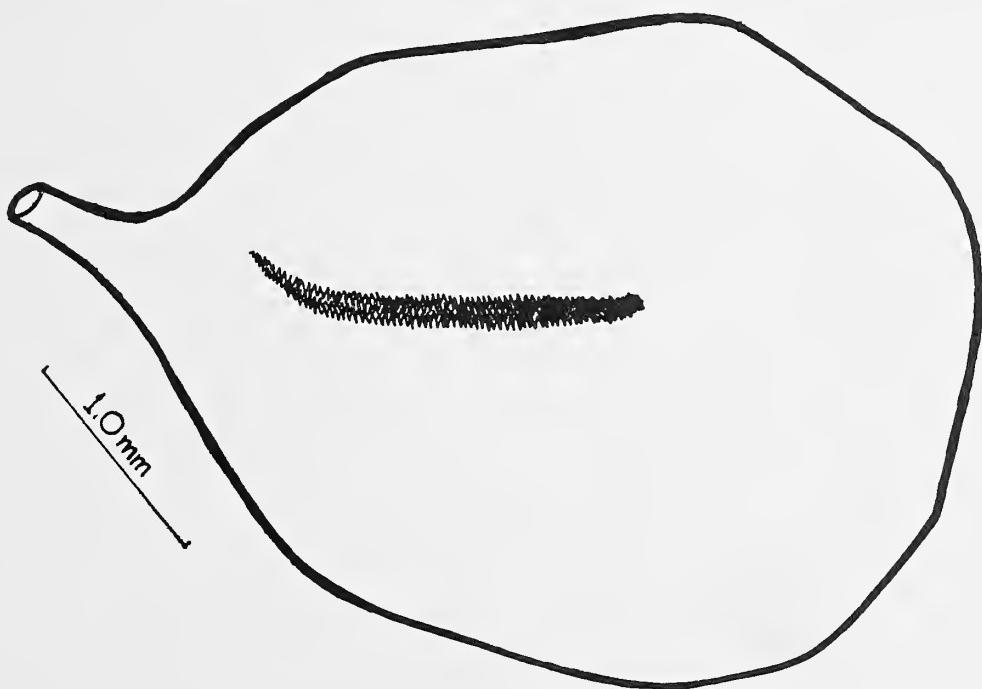
TEXT-FIGURE 2. *Heliconius nattereri,* female, bursa copulatrix.
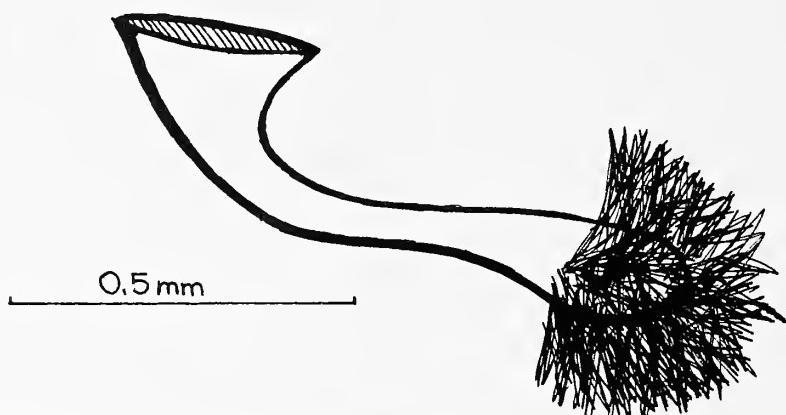
TEXT-FIGURE 3. *Heliconius nattereri*, female, abdominal process.

TEXT-FIGURE 4. *Heliconius nattereri*, female, specialized scale of abdominal process.

nychial processes of the metapretarsus are narrow, pointed, and subequal, as in members of the *melpomene*-group.

Heliconius nattereri seems to be a very primitive, inflexible, sensitive, and evidently declining species, with a rather uncertain future. We have tried many times to adapt it to life in a cage; no success has been obtained even with individuals hand-reared from eggs. We have introduced two *T. ovalis* plants into the largest of Dr. Augusto Ruschi's hummingbird aviaries (50 x 17 x 7 meters) in Santa Teresa, where a final attempt will be made to domesticate the species; any colony maintained there, however, would be at best very fragmentary and nonnatural. The species should survive in the various forest reserves in the Santa Teresa area, and the woods of the large colony visited in 1970 (Table 2) are owned by an enlightened conservationist who intends to preserve the area in the foreseeable future. The focus of this colony (areas D to L), however, is not on his property, and this area is presently in final stages of being preserved as a state biological reserve, through efforts of Dr. Ruschi with the state of Espírito Santo. Although this reserve may be one of the first to be erected primarily to preserve an endangered butterfly species, it is also very rich in a wide variety of plants, insects, birds, and mammals, and seems to be a funnel for insect migration as well. Seventeen of the 18 species of heliconian normally found in extra-Amazonian Brazil have been discovered breeding in this area, and the eighteenth (*Dione moneta*) may eventually occur as an invader from the west, since other western species, characteristic of the central plateau (such as *Tithorea harmonia pseudethra* and *Callicore sorana*) have been encountered in Santa Teresa.

Although intensive field collecting of *nattereri* is obviously inadvisable, Tables 1 and 2 help to support the conclusion that normal populations would not be unduly endangered by casual predation by man. Although the natural sex ratio is probably unity (see Table 3), only a quarter as many females as males have been observed in the field, even with extensive work near favored flowers and food-plants. Of these, less than half could have been captured, and of those which were captured, most had passed their principal egg-laying period. The principal natural control on *nattereri,* as with most heliconians, is surely ant and spider attack on eggs and young larvae. The additional factor of biological competition from other commoner and more adaptable species of *Heliconius* may help to weight the scales in favor of its decline in the present day. The principal threat to *nattereri* by man is surely the destruction of its virgin habitat, which is today essentially complete in vast areas of Bahia, Espírito Santo, and especially eastern Minas Gerais. The species does not seem to possess the flexibility to adapt to second growth or other grossly disturbed habitats.

Very partial cutting of the primeval forest may occasionally benefit *nattereri,* as it produces small clearings in which both *T. ovalis* and flower food can grow rapidly and prosper, giving conditions in which *nattereri* can compete with and survive in the presence of the five more advanced *Heliconius* species which use the same food-plant. However, as these five species have often adapted to secondary forests and more open areas created by man, where they employ other food-plants, they may be commoner today than in the past and thus far more dangerous to the future of *nattereri.* This applies even in the virgin forest where *T. ovalis* produces abundant meristems in clearings and along the steep gullies. Partial cutting of the forest is not often the method of land usage in *nattereri's* former range; most of the region, multiply razed and burned, has become today a sterile saw-grass desert.

From our field observations, we believe that at least a square kilometer of steep, humid, and food-plant-rich virgin forest is necessary for the persistence of a healthy colony of *nattereri.* Very few such tracts still exist in its former range, and many of those which do are falling to ax and to fire each year. Thus we regard *Heliconius nattereri* as among those inflexible and primitive forest-adapted animal species whose existence is presently placed in danger through the direct and indirect intervention of man.

EVOLUTION OF *Heliconius* AND *Eueides* SPECIES

The difficulty in objective correlation of all the facts presently available on the 55 species we have recognized in the genera *Heliconius* and *Eueides,* to develop a rational and ordered scheme for the radial evolution of these species, has been mentioned by Turner (1968b), with a suggestion that these data be analyzed by numerical taxonomy. The information and graph given by Emsley (1965), however, are so coherent with essentially all the known material, including that later published by Turner (1968b), that they seem to need only minor modifications to accurately represent the present state of knowledge of this very unusual and biologically useful group of butterflies.

The only information in the present paper which casts new light on the evolution of the two genera, the biology and revised morphology of *Heliconius nattereri,* is nevertheless significant enough to suggest one major reorganiza-

tion of Emsley's phylogeny. From the juvenile and adult biology of *H. nattereri,* the conclusion can be drawn that this species is only shortly removed from the principal evolutionary line between proto-*Dryas* and the silvaniform *Heliconius.* The egg and pupa of *nattereri* are barely distinguishable from those characteristic of species in the *silvana* and *melpomene*-groups. The larva shows its relationship to more primitive heliconians through its harlequin head-pattern and very long scoli (Beebe, Crane, and Fleming, 1960), but its overall appearance is very similar to that of larvae of members of the silvaniforms and *melpomene*-group, with, however, dark underparts as in members of the *erato* and *charitonia*-groups (Beebe, Crane, and Fleming, 1960; Turner, 1968b). The adult female, morphologically close to the silvaniforms, shows a primitive or proto-silvaniform color pattern; this in turn can be derived directly from the pattern of the adult male, by reversion of the color of the cubital bar on the forewing from light yellow to the presumably more primitive orange, and addition of narrow orange bars on the forewing inner margin and hindwing submarginal area. The adult male color pattern, as suggested by Emsley (1965), may be produced by simple substitution of yellow for orange in the most primitive heliconian pattern (present today in the pre-*Heliconius* species of *Agraulis, Dione, Dryadula, Podotricha,* and *Dryas,* and in *Eueides aliphera,* male *vibilia, lineata,* and *lybia*).

The orange color in *Dryas iulia* is at least partially composed of pteridines (Baust, 1967), but is probably principally oligo- or polymeric in nature (ommin or "melanin"); while the yellow pigment is 3-hydroxykynurenine, highly characteristic of and present in all species of the genus *Heliconius* (as well as in two other restricted groups in the Nymphalidae; see Brown, 1965, 1967; Brown and Domingues, 1970; Tokuyama *et al.,* 1967). The androconial distribution in the adult male of *nattereri* can be derived from that of *Dryas iulia* by simple loss of these specialized scales from the forewing cubitus; the genital valves are distinctly related to those of members of the *silvana*-group, and do not show the denticulate processes well developed in members of the more primitive groups of *Heliconius* (*hierax, aoede, wallacei, xanthocles, doris*) and in *Eueides* (see below). The female morphology only differs from that of members of the *silvana* and *melpomene*-groups by lack of a right-angle bend near the end of the signa on the bursa copulatrix.

The apparent intermediate position of *nattereri* between the more primitive heliconiine genera, especially but not exclusively *Dryas,* and the members of the *silvana, melpomene,* *erato,* and *charitonia* groups of *Heliconius,* with definitely close affinities to the silvaniforms and without clear relationships to *Eueides* or the more primitive *Heliconius,* leads us to propose a very ancient bifurcation in the evolution of these genera (Graph 3), probably occurring before the separation of the Central and South American land areas at the beginning of the Tertiary. We do not eliminate the possibility of a subsequent convergence not indicated in the graph, but also do not regard this as necessary to explain and order the data presently available.

One branch, which we regard as more primitive, includes the genus *Eueides,* probably with ten species; a small group of *Heliconius* probably including four species, in at least one of which the pupa strongly resembles those of *Eueides* species, with an abdominal flexure causing it to lie horizontally under the pupation surface, but which species have, in common with other *Heliconius,* 5-jointed female foretarsi and storage of 3-hydroxy-L-kynurenine as wing pigment; the *wallacei* and *xanthocles* groups of *Heliconius,* with four and two (or possibly three) species, respectively; and, as the most aberrant, and in our judgment most evolved member, *doris* in the proposed subgenus *Laparus* Billberg, 1820.

The other branch goes directly to the *nattereri* junction, independently and without relation to members of the first branch; it also undergoes a very early bifurcation, possibly even before giving rise to *nattereri.* The more primitive subbranch leads to the *silvana*-group, then through a transitional group of four species and finally to the *melpomene*-group, of which the most familiar member, *melpomene,* is probably the only species in the subbranch to use red forewing bands in courtship recognition. The second subbranch immediately bifurcates again, one arm leading to the *erato*-group (in which at least the species *erato* is red-responding), and the other arm leading through the *charitonia*-group and the *sara*-group to the most recent and evolved *sapho*-complex.

The alternative evolutionary process to that shown in Graph 3 would have the species of *Heliconius* arising from a single line posterior to *Eueides,* with *nattereri* following the appearance of the primitive groups in the scheme of the Graph. This would require the independent convergent evolution of extremely similar pupae in *H. aoede* and *Eueides,* simultaneous with strongly and rapidly divergent evolution of the *nattereri* and *silvana*-groups. This possibility is viable and cannot be eliminated; it would be supported by the evident convergent evolution which has produced nearly identical fifth-stage larval color patterns in the most advanced

Eueides (*E. tales*) and the silvaniform *Heliconius* with which it shares food-plants (K. Brown, unpublished observations). Acceptance of this alternative would merely require linking the junction of the *wallacei* and *xanthocles* groups with the *nattereri* and *silvana*-groups above them, which would no longer be linked directly to the line arising after *Dryas iulia*. The scheme in Graph 3 has the advantage of requiring convergent evolution in but two characters which also appear in groups other than *Heliconius* by independent processes: a lower chromosome number is also present in the most primitive heliconian genus, *Philaethria*, and is coincidentally (?) equal to that most widespread in *Heliconius* (21); and 3-hydroxy-*L*-kynurenine storage is independently acquired by most genera of the Ithomiinae and by females of *Catonephele* in the Nymphalinae (Brown and Domingues, 1970).

In the final arrangement of Graph 3, we have attempted to give significance to the placement of the species names, setting postulated geohistorical isochronals at about 45° right. While not necessarily implying a fundamental conservatism in *Heliconius* wing-patterns in the very long period since their initial formation, the graph accepts the probability of simultaneous acquiral of certain patterns by morphologically diverse members of the group, and subsequent stabilization by normal reinforcement of selective advantage through Müllerian mimicry. Thus, the first species to develop the black-yellow-red dennis-rayed pattern, possibly simultaneously with the formation of a colonizable Amazon Basin in the Miocene, are considered to be *Eueides tales* and *eanes* and *Heliconius aoede*, *burneyi*, and *xanthocles*, one derived from each primitive group in the two genera; the very similar pattern worn by the female of the upper Amazonian *Eueides vibilia unifasciatus* may have developed more or less simultaneously, or during an isolation of this area during the Pleistocene weather changes. This pattern may have been originally stabilized through rough resemblance to common black, yellow, and orange ithomiines (Emsley, 1964); these highly distasteful and successful primitive Nymphalidae surely inspired the mimetic patterns of *Eueides isabella* and *lampeto* and the silvaniform *Heliconius*, and probably of the females of *Eueides vibilia vibilia* and *E. v. vialis* and *Heliconius nattereri*.

The characteristic heliconian pattern of a yellow forewing band and a broad red basal patch on the hindwing was first achieved by the very primitive species *Eueides procula* and *Heliconius hierax*, then by *H. egeria* (nominate form) and the most primitive member of the

erato-group (*H. himera*), and finally by the advanced species *H. clysonymus*, *hortense*, *ricini*, and *demeter* (males only). Though sympatry among the six species which bear this color-pattern monomorphically (*egeria* and *demeter* also produce dennis-rayed forms and probably are better linked to this other color-pattern-group) is rare, it is not unknown as was implied by Emsley (1965); indeed, *H. clysonymus* probably flies in some part of its range with every other member of the group* except *hortense*, a northern and possibly still interfertile splinter of *clysonymus* itself. A very widespread pattern in *Heliconius*, the iridescent blue (or black) ground-color with yellow or white forewing bands, is regarded as more recent than the above two patterns (Emsley, 1965) but older than the dennis-rayed pattern in the Amazon area; it is rarely shown by any of the more primitive Nymphalid groups. First achieved by *Heliconius metharme* and *H. wallacei* (which also possess on the ventral hindwing surface both a white rayed pattern, in common with their predecessor *H. hierax*, and a variable primitive red ray pattern, as in their followers *H. aoede* and *H. burneyi*), the pattern was next adopted by some subspecies of *Eueides eanes*, by *H. luciana* and the nominate form of *H. timareta* (which also exists in sympatric dennis-rayed and yellow forewing band—red hindwing basal patch forms, thus acquiring protection by three separate mimetic associations), to reach culmination in the advanced species *H. sara*, *H. leucadia*, *H. antiochus*, and *H. congener*. Many subspecies of *H. cydno* and a few of *H. sapho* also show this pattern, while the remaining subspecies converge either toward each other or to a separate pattern (as in *cydno hermogenes*, to *hecalesia*, or *cydno gustavi*, to *erato chestertonii*).

The pattern elements of *H. besckei* probably appeared more or less simultaneously in *H. hermathena* and *H. telesiphe*, the northern *H. erato petiverana* and *H. melpomene rosina*, the central-western *H. erato favorinus* and *H. melpomene amaryllis*, and the southern *H. erato phyllis* and *H. melpomene nanna* and *amandus*; the populations of these latter two species were then separated when their Amazonian subspecies adopted the dennis-rayed pattern. Additional heliconian color-patterns seem closely linked to those of more primitive nymphalids in their respective areas: *Heliconius charitonia*, *atthis*, and *hecuba* to *Elzunia* species; *Eueides procula edias* and *Heliconius godmani*, *hecale-*

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* *Heliconius clysonymus* intergrades with *H. c. hygiana* in western Colombia (see Part II), and is sympatric with *Eueides procula* and *H. ricini* in north-central Venezuela; with *H. hierax* along all the eastern slopes of the Colombian and Ecuadorian Andes; and probably with *H. himera* in southeastern Ecuador.
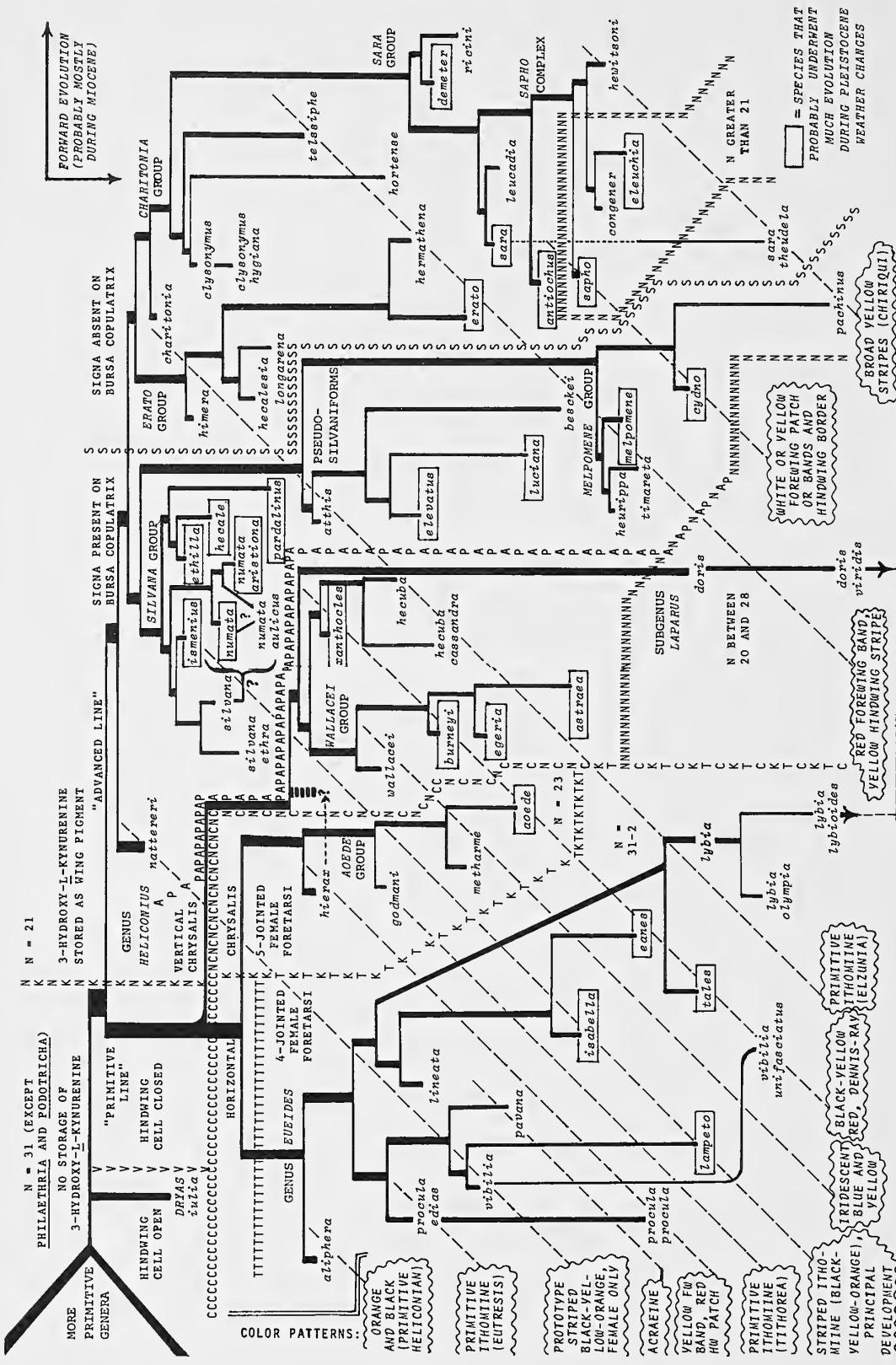
GRAPH 3. A working model for the possible evolutionary history of the fifty-five species of *Heliconius* and *Eueides*. Solid lines represent the dendrogram of presumed taxonomic relationships. Extra-heavy vertical lines denote well-marked species or groups; these follow the divisions of Emsley (1965) in most cases.

The placement of the names of the species is significant; geohistorical isochronals are at about 45° right. The general arrangement follows the evolutionary discussion in Emsley (1965) and additional published information, including data in this paper and in Part II. The species *nattereri*, though derived from the "advanced line" of the genus *Heliconius*, is regarded as the most primitive in this genus, of similar antiquity as *Eueides vibilia*. A few species which have apparently undergone much evolution, with the formation of well-separated subspecies, since their presumed first appearance (*Eueides procula* and *vibilia*, *Heliconius doris*, *numata*, *clysonymus*, and *sara*) are represented over a range of geological time; others, which could not thus be represented due to space limitations on the Graph, are indicated by boxes. A very large number of subspecies, and conceivably a few species such as *pardalinus* or *leucadia*, were surely formed during the Pleistocene weather changes in the Amazon Basin.

Taxonomic uncertainties (see Part II of this series) are represented as possibly not interfertile subspecies either below or beside the parent species: *Eueides procula procula*, *lybia olympia* and *lybia lybioides*, and *Heliconius hecuba cassandra* and *silvana ethra*.

On the left and lower margins are indicated, in capital script, a total of thirteen color-pattern types in the two genera. Dotted lines emanating from these pattern descriptors at 45° right cross the species in which the pattern may have first appeared; see discussion in the text.

Important characters which form major systematic divisions for the fifty-five species are indicated on the Graph, with their divisions marked by rows of letters derived from these characters (for example, CCCCC for horizontal versus vertical chrysalis, and NNNNN for difference in chromosome numbers). The following comments and references apply to these characters as used in the elaboration of the Graph:

Chromosome numbers: de Lesse, 1967, 1970a, 1970b; de Lesse and K. Brown, in press; Soumalainen, Cook, and Turner, 1971; T. Emmel, personal communication; and K. Brown, T. Emmel, and Soumalainen, work in progress. Uncertainties exist in the reported numbers for the *sapho* group; all species have been refixed, and are awaiting counts. None of the four males of *sapho* from Santo Domingo which were dissected had an aberrant (doubled) testicle as did the individual fixed by de Lesse from the identical population. In the more primitive members of the genus *Heliconius*, only *aoede* and *wallacei* have known numbers; we have fixed *hierax*, *godmani*, *metharme*, five subspecies of *aoede*, *burneyi*, *astraea*, *nattereri*, and four subspecies of *silvana*, which are awaiting counts.

3-Hydroxy-*L*-kynurenine: Brown and Domingues, 1970.

Chrysalis position: Beebe, Crane, and Fleming, 1960; Turner, 1968b; and K. Brown and W. Benson, unpublished observations. The position is uncertain for *Heliconius hierax*, which may fall outside of (though not necessarily subsequent to) the line including *Eueides* and the *aoede*-group; this uncertainty is indicated in the graph. Of the three species in the *aoede*-group, only *aoede* has the chrysalis known (Turner, 1968b), though the other two species can be presumed similar through consideration of the extreme similarity of the morphology of the adults.

Female foretarsi and signa: Emsley, 1965.

"Primitive" and "Advanced" lines: this paper.

sia, and *longarena* to species of *Athesis, Eutresis, Olyras,* and *Tithorea* (these five are all very primitive ithomiine genera); and *Eueides pavana* to *Actinote* species (Acraeinae). The final pattern, probably developing on the isolated island of Talamanca, formed at the end of the Miocene and probably relinked to the continent only in the Pleistocene, is the black-and-yellow striped appearance of *Heliconius pachinus, hewitsoni, sara theudela,* and *doris viridis.* The isolated orange-striped subspecies *Eueides lybia lybioides* was probably also produced during this period.

Two species of *Heliconius* are rather puzzling in their evolutionary relationships: *H. hermathena* and *H. telesiphe.* The first is extremely rare and little-known, though evidently found sparsely in all the northern half of the Amazon Basin from Belém as far west as Caquetá, Colombia. Except in the mimetic form *vereatta,* from Faro between Obidos and Manaus, which has lost all yellow stripes from the upper wing surface, it possesses an absolutely unique color-pattern, with probably very low potential for survival through mimetic association. The adult morphology, egg, and young larva of *hermathena* suggest a close relationship to *H. erato,* from which it may be a "splinter species" of ancient lineage, maintaining a primitive *erato/ charitonia* type color-pattern to the present day. From afar, *hermathena* looks very much like *erato petiverana* or *e. phyllis.* It occasionally flies together with polymorphic red-banded (but never also yellow-striped) populations of *erato* and *melpomene* near Faro, Obidos, Santarém, and Maués. It is more commonly sympatric, however, with dennis (-rayed) *erato* near Belém, Manaus, Manicoré, São Gabriel (upper Rio Negro), and in southern Colombia. In these latter areas it can have no possible Müllerian association with other *Heliconius,* and apparently persists through a variety of other protective devices. Observations made on an extremely restricted colony near Manaus (K. Brown and W. Benson, in preparation) suggest that it will be found to occur only in "campina," low-vegetation (*cerrado*) pockets on deep coarse sand within the Amazon Basin. Here it feeds on an abundant primitive *Passiflora (P. [Astrophea] faroana)* which is also restricted to this unusual and infrequent biotope. The adults fly in the shade near the ground under the sparse, twisted trees, and have a rapid, linear, and jerky flight somewhat reminiscent of that of the (also non-mimetic) *H. charitonia.* Like this latter species, they dodge and disappear in the poor dappled light very effectively when threatened. Their hot, sandy habitat is quite unattractive to bird predators, in any case. The small young vines chosen by the females for oviposition do not seem to attract ant, lizard, or avian enemies

to the juvenile stages; however, one potentially severe source of larval mortality is Tachinid parasites. A variety of further ecological specializations have surely contributed to the unexpected survival of this most singular non-mimetic species to the present time; for example, the older larva does not resemble those of its near relatives or of any other *Heliconius* species, but converges strongly on the color-pattern of sympatric *Philaethria* larvae, feeding on larger *P. faroana* vines. Perhaps the brightest hope for the future of this highly specialized relict species is represented in the form *vereatta,* an apparently not uncommon and very effective *erato hydara* mimic known (with transitions to the nominate form) so far only in Faro, from where *P. faroana* also was described.

Heliconius telesiphe, which flies to over 2500 meters elevation in the Andean montane forests, is undoubtedly closely related to *H. clysonimus;* its androconial distribution, male genital valves, and straight, broad female abdominal processes indicate that it and *clysonymus* (with its subspecies, *c. hygiana,* also possessing straight female abdominal processes) should be placed in the *charitonia*-group, not far removed from the bifurcation which led to the *erato*-group. Like *hermathena* and *erato, telesiphe* has developed red instead of yellow forewing bands, possibly used in color-recognition in courtship. Northern and extreme southern populations of *telesiphe* (Colombia and Ecuador, and Santa Cruz, Bolívia) have a yellow hindwing band, as do sympatric populations of *Podotricha telesiphe* and *Heliconius erato* and *melpomene,* while intermediate populations have a white hindwing band and fly together with *erato* and *melpomene* subspecies which lack yellow hindwing bands; exceptions to this generalization are known in central Ecuador and in central Peru. The flight behavior of *telesiphe* and its preference for montane forest probably indicate that it derives little from mimetic association with *erato* and *melpomene.*

An Explanatory Addition on Materials and Methods

The placement of the species in Graph 3, as well as the discussions in this paper, are based on the following assumptions, some of which have already been indicated in the divisions of the graph:

(1) The genus *Eueides,* with $N = 31$ and no storage of 3-hydroxy-*L*-kynurenine, is more closely allied to primitive genera (which share these characters) than is *Heliconius,* and it is a terminal offshoot (that is, no members of *Heliconius* have developed from members of *Eueides*).

(2) The *doris* and *sapho* groups of *Heliconius,* with variable chromosome numbers and gregarious larvae, are the most advanced.

(3) The loss of the signa on the bursa copulatrix, and the reduction of the ventral paronychial processes on the female pretarsi, are to be regarded as advanced characters in *Heliconius.*

(4) Evolution is accompanied by progressive loss of androconia from male fore- and hindwing veins, and/or scattering of these specialized scales on the hindwing membrane.

(5) Gradual and progressive changes in the shape of the male genital valves and female signa are frequently observable in the evolution of the species, and may be assigned correlative significance. Likewise, marked similarity in these characters and the shape of the female abdominal processes between two different species is likely to be significant and not coincidental.

(6) However, appreciable intraspecific variations in male genital valves, androconial distributions, female abdominal processes, and forms of early stages have been shown to be possible

in *Heliconius* and *Eueides,* and must be judged in connection with other evidence (especially field sympatry with or without intergradation) in uniting or separating species in this group. A basically biological, rather than strictly morphological, definition of the species is held to be the most veridical in cases of structural or evolutionary ambiguity.

As in the second part of this series, the author has examined over a dozen major and essentially complete *Heliconius* collections (including over 10,000 prepared specimens in the Museu Nacional, Rio), and several dozen partially complete and complementary collections, and has undertaken extensive studies of sympatry, hybridization, and juvenile biology in the field, in the development of the scheme represented in Graph 3. Some tentative comparisons of behavioral characteristics which can be observed in relatively more primitive and relatively more advanced members of various groups of heliconians, based on collated field data from many sources, are presented in Table 4, with examples of some species with which we have extensive experience in th field. Although the

TABLE 4. FIELD BEHAVIOR OF HELICONIANS

Some generalizations about relatively primitive and relatively advanced species, based upon extensive field observations of the species mentioned in the examples.

	"PRIMITIVE" SPECIES	"ADVANCED" SPECIES
Geographic distribution	Tend to be narrowly restricted to a single faunal region	Widespread, overlapping many faunal regions
Microecological preference	Restricted to primary forest (or rarely to another biotope)	Found in a wide variety of habitats, including those created by man.
Behavior analysis (in dichotomous pairs)	Strongly fixed behavior	Adaptable, flexible behavior
	Large-scale promenading by males	Little or no promenading
	Males do not tolerate each other	Males more tolerant of each other
	Relatively high-flying, in forest	Relatively low-flying, often encountered on edges or in open
	Females shy and retiring, very rarely observed even on flowers	Females more open and fearless, frequently observed on flowers
	Flower-visiting restricted to set hours, infrequent, brief	Flower visiting often all day, frequent, extended
Most typical examples (relationships are in horizontal; vertical relations are less certain)	*Eueides pavana, vibilia, lineata* *Heliconius*	*isabella, tales*
	1st branch *hierax, xanthocles, wallacei*	*doris*
	2nd branch *nattereri, silvana, ismenius*	*ethilla, hecale, melpomene*
	3rd branch *hecalesia*	*erato, clysonymus*
	4th branch *telesiphe, demeter*	*sara, charitonia, antiochus*

Note that most species fall between the two stereotypes; however, the proximity to each may be judged from this Table and could be useful in assigning evolutionary position.

majority of species are intermediate within this artificial behavioral dichotomy, and the most primitive members of the more advanced groups are perhaps still more towards the "advanced behavior" side of the dichotomy than the most advanced members of *Eueides,* the characters noted in Table 4 correlate quite well with morphological, biological, and distributional data known on the species. Hopefully, the table will be of assistance in future studies designed to refine and modify the working model for evolution presented in Graph 3.

SUMMARY

(1) The ecology and biology of *Heliconius nattereri,* a key primitive species apparently nearing its natural extinction, whose uniquely dimorphic female has been known heretofore as *H. fruhstorferi,* are described and discussed.

(2) On the basis of published and new morphological and biological data and extensive field observations, and in view of the apparent phylogenetic position of *H. nattereri* between *Dryas iulia* and *Heliconius silvana,* a modified graph of the geohistorical evolution of the 55 species of *Heliconius* and *Eueides* is presented and explained.

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EXPLANATION OF THE PLATES

PLATE I

FIGURES 1-6. *Heliconius nattereri* in nature, Santa Teresa, Espírito Santo, life size. Males black and yellow, females black, yellow, and orange; basal dots red.

1. Typical male on Poinsettia, dorsal.

2. Yellow-bar female on *Lantana,* dorsal.

3. Female with convergent forewing bars, dorsal.

4. Orange-bar female on blue *Eupatorium,* dorsal.

5. Male on *Lantana,* ventral.

6. Female on *Passiflora kermesina,* ventral.

FIGURE 7. *Dismorphia astyocha* Hubner, 1827-1831, Santa Teresa, Espírito Santo, ventral, life size. Black, yellow, and orange.

FIGURE 8. *Dismorphia astyocha,* female on *Eupatorium,* Santa Teresa, dorsal, life size.

FIGURE 9. *Perrhybris flava* Oberthür, 1896 (male above, female beneath), Santa Teresa area, Espírito Santo, dorsal, life size. Male black and yellow, female black, yellow, and red-orange.
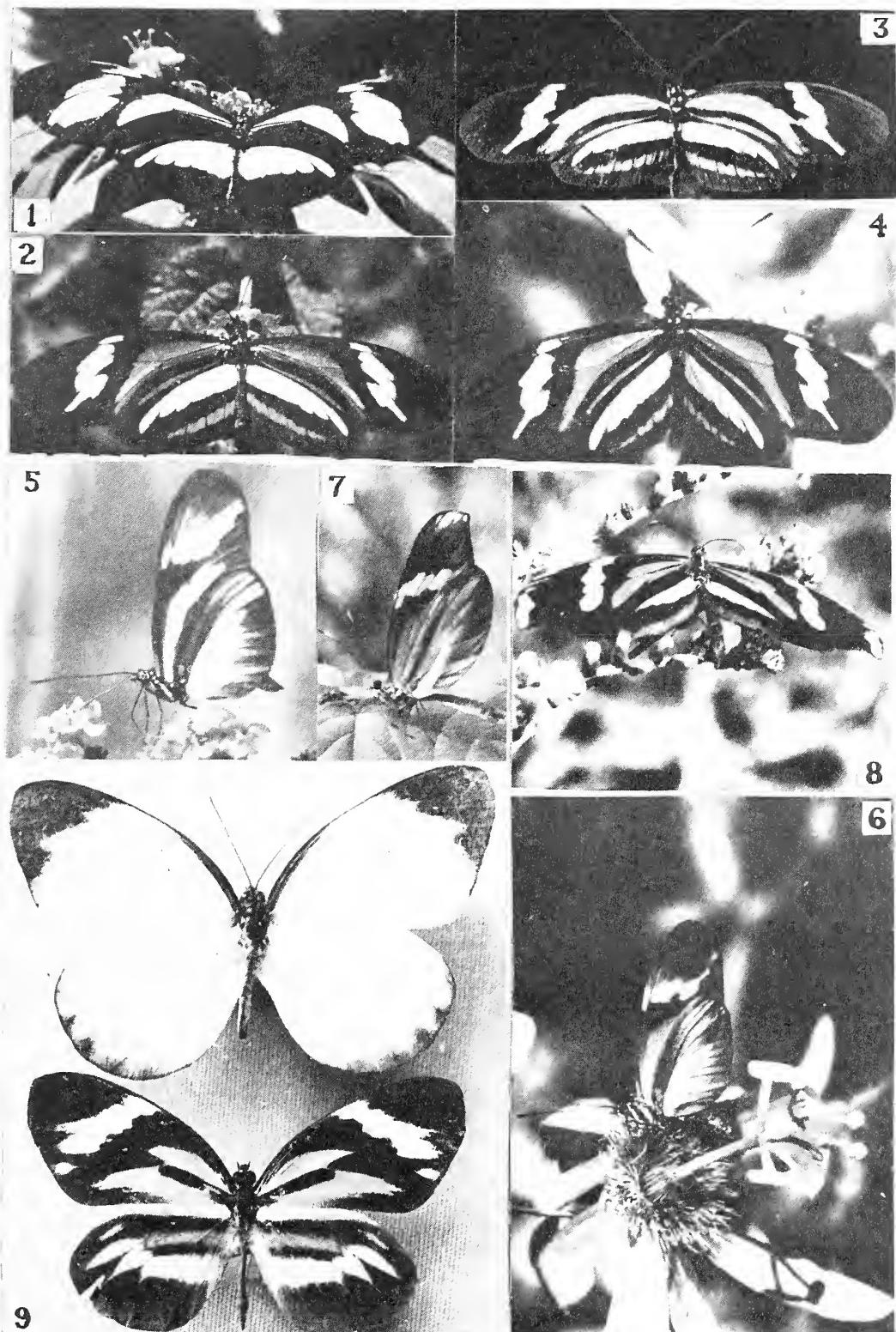
PLATE I

PLATE II

FIGURE 10. Upper left: *Phyciodes lansdorfi* (Godart, 1819), typical female, Barbacena, Minas Gerais.

Upper right: *Phyciodes lansdorfi*, transitional female near form *jacinthica* Röber, 1914, Rio de Janeiro.

Middle left: *Phyciodes lansdorfi*, mimetic female very near *jacinthica*, Santa Teresa, Espírito Santo.

Middle right: *Phyciodes eunice esora* Hewitson, 1857, male, Conceição da Barra, Espírito Santo.

Lower: *Phyciodes erysice* (Geyer, 1832), male, Uruçuca, Bahia.

All dorsal, life size. Black, yellow, and orange.

FIGURE 11. Ithomiinae and Dysschematidae mimetic of male *nattereri*. All dorsal, life size. Black and yellow or ochre.

Upper left: *Aeria olena* Weymer, 1875, Conceição da Barra, E.S.

Upper right: *Scada reckia* (Hübner, 1828), Ubatã, Bahia.

Middle left: *Napeogenes yanetta* (Hewitson, 1867), Santa Teresa, E.S.

Middle right: *Napeogenes sulphurina* Bates, 1860, Conceição da Barra.

Center: *Phaloë cruenta* (Hübner, 1819-21), Itatiaia, Rio de Janeiro.

Lower left: *Notophyson swainsoni* (Druce, 1895), Rio de Janeiro.

Lower right: *Ephestris melaxanthe* (Hübner, 1809), Teresópolis, R.J.

FIGURES 12-16. *Heliconius nattereri,* juvenile stages, Santa Teresa, E.S.

12. Egg, twenty times life size. Yellow.

13. Mature first instar larva, 10x. Deep yellow-brown.

14. First to second instar molt, 10x.

15. Second instar larva, dorsal, 6x. Dark greenish-brown.

16. Second to third instar molt, 6x.
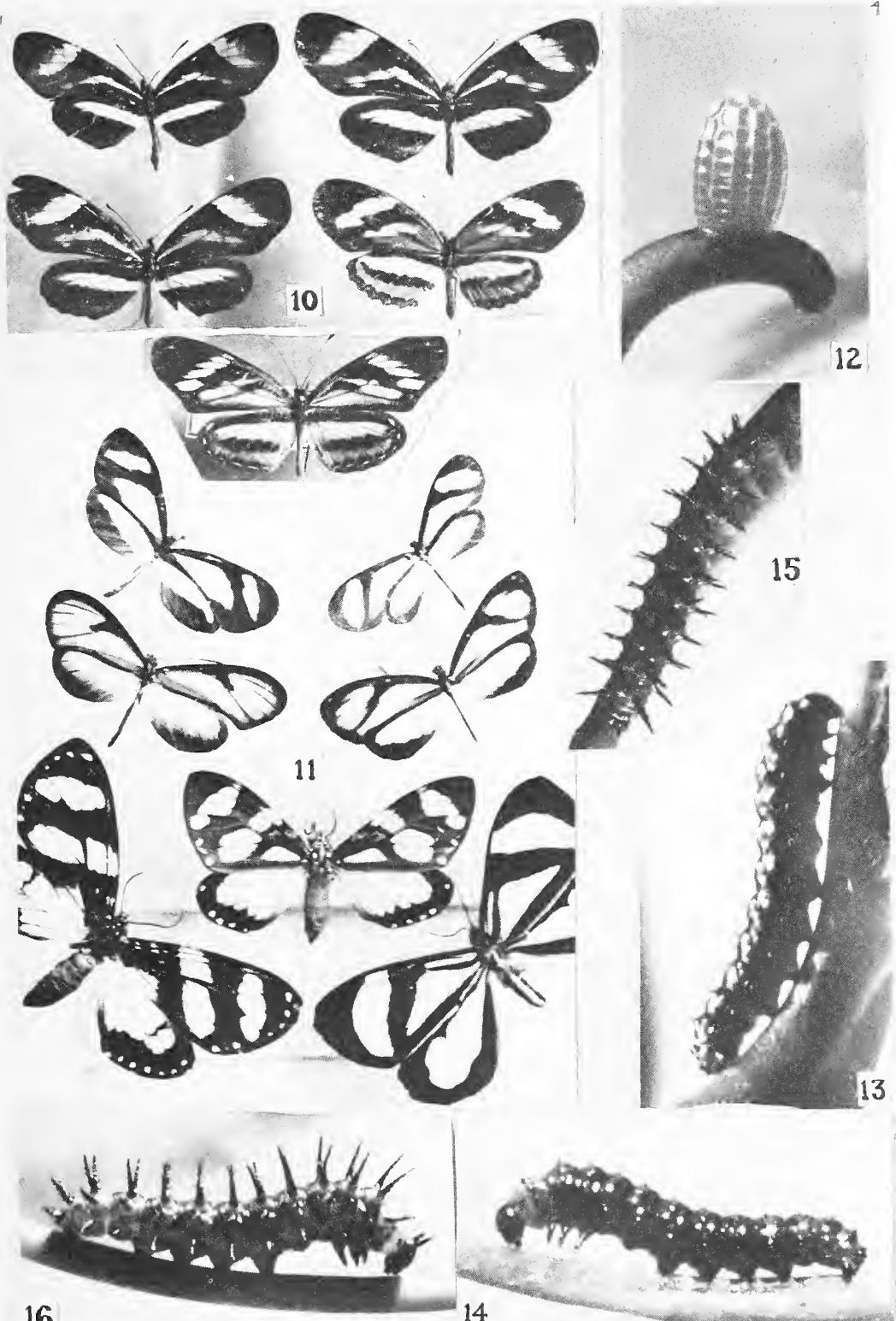
PLATE II

PLATE III

FIGURES 17-30. *Heliconius nattereri*, juvenile stages, Santa Teresa.

17. Third instar larva, 4x. Light greenish-brown.

18. Light fourth instar larva, 2x. Greenish-white, black.

19. Dark fourth instar larva, detail of abdomen. 5x.

20. Mature larva, 3x. Black and white, yellow head.

21. Fourth instar larva, detail of head pattern. 5x.

22. Mature larva, detail of head and thorax. 3.5x.

23. Mature larva, front view of head. 3x.

24. Mature larva preparing to pupate. Life size.

25. Dark pupa, laterodorsal, twice life size.

26. Light pupa, laterodorsal, twice life size.

27. Dark pupa, dorsal, twice life size.

28. Dark pupa, ventral, twice life size.

29. Dark pupa, lateral, twice life size.

30. Light pupa, lateral, twice life size.
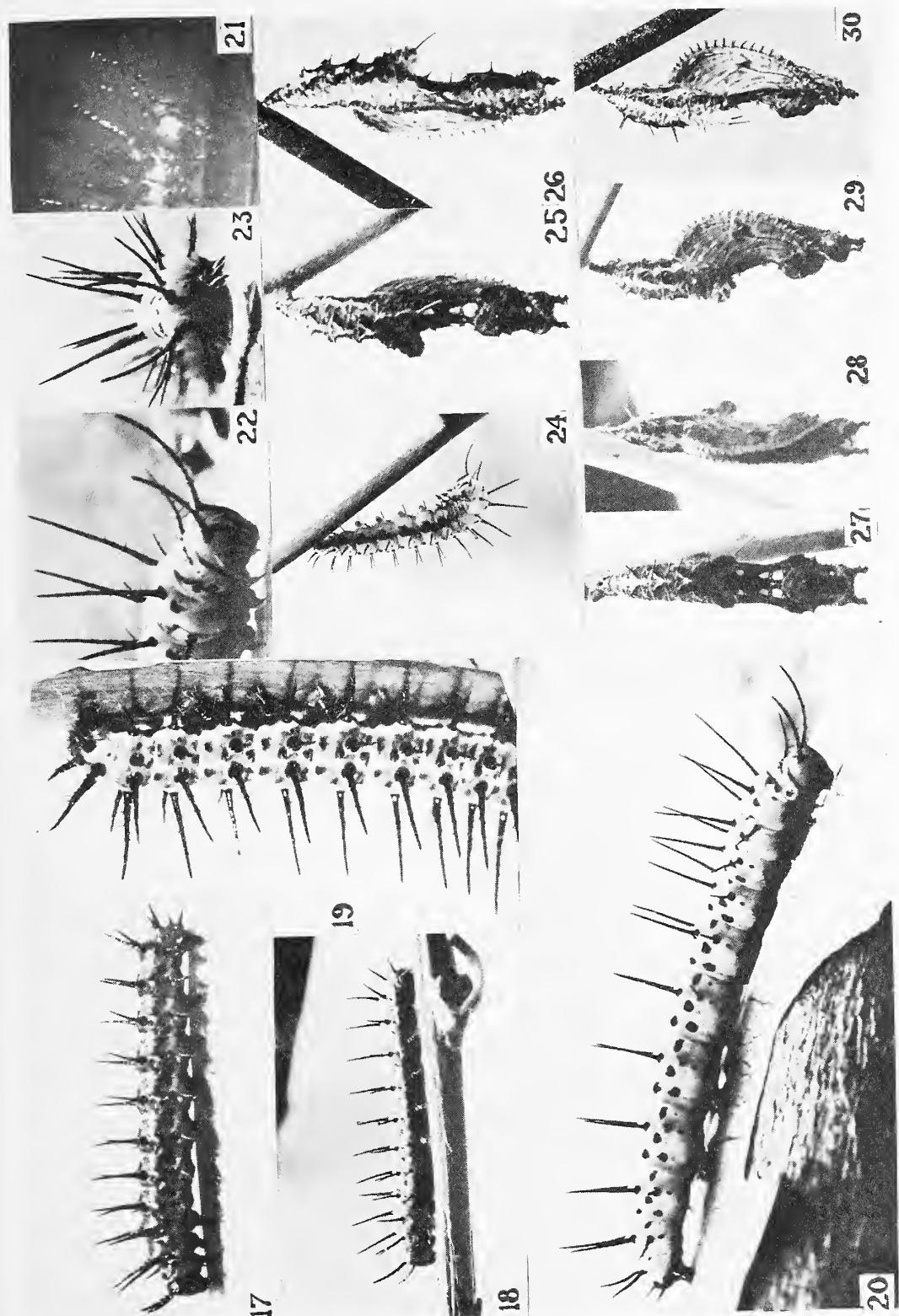
PLATE III

PLATE IV

FIGURES 31-37. Emergence of a female imago of *Heliconius nattereri* from the pupa. Early morning, Rio de Janeiro (bred through from an egg obtained in Santa Teresa). All life size. Black, yellow, and orange.

time
scale
(min. sec.)

0.05 31. Dark phase female pupa: wing case beginning to split.

0.15 32. Wing case and antenna case separating under leg pressure.

0.25 33. Body beginning to pull itself downward and forward.

0.40 34. One wing free, body continuing to pull down and out.

1.00 35. Imago free from pupa case, wings beginning to expand. Palps vibrating forward and outward.

3.00 36. Wings nearly expanded. The delicate task of joining the separate halves of the proboscis to form a tube.

6.00 37. Antenna brought outside the fully expanded wings.

FIGURE 38. Light phase male pupa one hour before emergence, showing general darkening of color (except for the abdominal flanges) and revealing the adult forewing pattern through the wing cases. Life size.

FIGURE 39. Adult male thirty minutes after emergence from the same light phase pupa. Life size. Black and yellow.

31 32 33

34 35 36

37 38 39

PLATE IV

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA

CONSELHO NACIONAL DE GEOGRAFIA

BRASIL

ESCALA 1:

RORAIMA · hydara

AMAPÁ · estrella

reduotimacula

AMAZONAS
typical
hybrid:
lativitta

PARÁ · amazona

MARANHÃO

CEARÁ · RIO GRANDE DO NORTE

PARAÍBA

PERNAMBUCO

PIAUÍ

ALAGOAS

SERGIPE

anna

ACRE

RONDÔNIA

MATO

GOIÁS · phyllis

BAHIA

vanustus

GROSSO

MINAS GERAIS

ESPÍRITO SANTO

SÃO PAULO

RIO DE JANEIRO

GUANABARA (city of Rio de Janeiro)

PARANÁ

SANTA CATARINA

RIO GRANDE DO SUL

LEGEND

Unpatterned area is extra-Amazonian.
Cities and collecting areas are
indicated by heavy dots.

▬▪▬▪ State boundaries

Southeastern mountain divide

Patterns in the Amazonian area indicate areas
occupied by the illustrated and named subspecies
of Helioonius erato.
Overlapping of two or more patterns indicate
probable locations of bland or hybridization
zones (see Figures 63 and 64).
Crosses in squares are points from which a
usable statistical sample of erato is available.

(RJ) Rio Cabaçal - Rio Branco - Rio Jaurú area: a south-
ward and eastward extension of the Hylaea (general
Amazonian forest), where H. numata variations, H.
silvana mirus, H. burneyi, H. leucadia and H. acede
subsp. nov. (see Part IV) fly in the Paraguay basin.

(CO) Farthest south confirmed locality for the "Cuyaba-
Corumba River System" species: H. demeter eratosig-
nis, H. rioini, H. elevatus schmassmanni, and H.
astraea subsp. nov. (see Part IV)

[CO] Poorly explored areas where these same four forms
may eventually be found in the Cuiabá drainage.

B   Areas where besokei and melpomane are sympatric.

C   New subspecies of ethilla (see Part IV).

D   Philaethria dido and P. werniokei sympatric.

E   H. ethilla eucoma in extra-Amazonian region.

I   Eueides isabella isabella outside the Amazon Basin.

J   Pure populations of Dione juno euffumata.

L   Known localities for Helioonius luciana.

M   Brazilian localities for Dione moneta moneta.

N   Heliconius nattereri seen in this century.

R   H. ricini outside the Amazon Basin.

T   H. sara thamar in extra-Amazonian Brazil.

U   Eueides vibilia unifasciatus outside Amazon Basin.

W   Helioonius wallacei flavescens outside Amazon area.

X   Heliconius xanthocles melete outside Amazon area.

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Contents

ZOOLOGICA

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3

The Hematological Parameters and Blood Cell Morphology of the Brown Bullhead Catfish, *Ictalurus nebulosus* (Le Sueur)

(Tables 1-3)

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The present research indicates that the various procedures utilized in the study of the hematopoietic systems of the higher classes of the vertebrates are applicable, with some modifications, for the study of the hematology of the poikilotherms. A description is presented of the peripheral blood cell constituents, with an emphasis on the distinction of the various stages of development of the erythrocytic and leucocytic series, reflected by: changes in the nuclear chromatin, location, size and shape of the nucleus, size and shape of the cell, and the state of cytoplasmic basophilia.

One of the aims of this study was to examine some aspects of the erythrocytic system in the normal, bled, and mechanically stressed bullhead catfish. Values were determined for red cell, white cell, reticulocyte, differentials, hematocrit, hemoglobin, and the erythrocyte corpuscular constants. Statistical analyses indicate a significant difference in the red blood cell count, white blood cell count, reticulocyte count, and mean corpuscular volume after bleeding. The evidence supports the view that there is a physiological control, possibly hormonal in nature, responsible for blood cell formation in different species of fish.

Introduction

THERE IS A NEED for additional information concerning the morphological and physiological characteristics of the blood of different fishes. The suitability of the catfish as an experimental animal in hematology and immunology has been clearly demonstrated in investigations conducted by Dawson (1935) and more recently by Chuba, *et al.* (1968); Wiener, *et al.* (1968); Kuhns, *et al.* (1969); and Baldo and Boettcher (1970).

The purpose of the present investigation was to obtain more quantitative data relating to the hematological parameters and morphology of catfish blood. Careful selection was made of methods previously used and in some cases a few modifications were instituted to improve upon specific procedures.

The blood cell values obtained from these fish may reliably be considered as representing the normal blood picture of the brown bullhead with the question as to a possible difference from wild catfish being unlikely. In this connection, it has been demonstrated that there were no significant differences in the numbers of erythrocytes, hematocrit percentages, and hemoglobin levels between the blood of wild and hatchery-reared lake trout (Piper and Stephens, 1962) and silver salmon fingerlings (Katz and Donaldson, 1950).

Materials and Methods

Aquaria and Fish

A normal group of brown bullhead catfish ranging in size from six to eight inches and weighing less than 50 grams were maintained in fiber glass tubs. A 365-gallon tub was found to be sufficient to support 30 brown bullhead catfish, if it was equipped with a filter system and water pump to maintain a constant flow of water, and two air lines for constant bubbling of air through the closed water system. The temperature, oxygen and pH of the aquaria were measured and recorded daily in the morning.

The temperature was maintained between 22° to 25° C, the oxygen content was above 5 ppm, and the pH was maintained between 6.3 to 8.5. These levels have been recommended by several fish hatcheries (Bureau of Sport Fisheries and Wildlife, 1970; Darragh Company, 1970; and Ralston Purina Company, 1970). The catfish were maintained on a diet of freshly ground beef, and fed daily in the morning. However, the fish were starved for a period of at least 24 hours prior to handling, either for the purpose of mechanically tagging, transfer to an experimental 50 gallon tank, or blood sampling. Even though sex determination of catfish is difficult, an attempt was made following descriptions outlined by some commercial fish journals (American Fish Farmer, Bureau of Sport Fisheries and Wildlife, 1970).

Blood Letting

The unanesthetized bullhead was placed ventral side up, held gently but securely by pressing down the abdomen, just below the pectoral fins. A 0.5 or 1.0 cc tuberculin syringe fitted with a one inch 20-gauge needle, premoistened with a 3.8 percent solution of sodium citrate in Locke's isotonic saline, was oriented with the ventral aorta and inserted gently into the heart, just beneath the pectoral girdle. The syringe plunger was gently pulled back until the desired quantity of blood was obtained. If sufficient blood was not obtained within 30 seconds after the initial penetration, the syringe was withdrawn and the fish was returned to the aquaria. Coagulation of blood, which porbably occurs in the pericardial cavity, could prevent the drawing of the blood sample through the syringe needle (Chuba, *et al.*, 1968; Klontz, 1968; and Dupree, 1970). For repeated blood samplings of fish, blood letting from the heart is recommended (Dupree, 1970). Bleeding from the caudal fin introduces the danger of destroying the caudal vein and bacterial infection. Bleeding was always performed during the same time in the morning to avoid the complication of possible diurnal variations.

It is important to note that 3.8 percent sodium citrate was found to be the only anticoagulant that did not destroy the cell morphology as seen in the blood smear preparations. Following the cardiac blood letting method outlined, fish can be bled from 0.25 ml to 2.0 ml with no mortality.

Determination of Hematological Parameters

Absolute blood cell counts. Erythrocyte counts were made by diluting one part blood with 200 parts of Hayem's solution in a red blood cell diluting pipette, counting the cells in the five smaller squares in a Spencer Bright-line hemocytometer, and multiplying the total count by 10^4 (Smith, *et al.*, 1952; Hesser, 1960; and Klontz, 1968). For the white cell count Shaw's Avian solutions were used (Hesser, 1960; and Klontz, 1968). Both solutions must be filtered prior to use. Blood was drawn up to the 0.5 mark of a red cell diluting pipette; Solution A was added to fill the bulb of the pipette approximately one-half full and mixed. Next, the pipette was filled to the 101 mark with Solution B. A hemocytometer was used and the cells in the four large squares were counted and multiplied by 500. All the counts were made in duplicate for each fish and averaged. The cell counts are representative for each cu mm of whole blood.

Hematocrit determination. The hematocrit was determined by the microhematocrit technique (Larsen and Sneiszko, 1961a). Blood was collected in commercially prepared heparinized capillary tubes and then centrifuged at a high speed for five minutes. Each pair of tubes for each fish was examined to determine the volume of packed red blood cells.

Hemoglobin concentration determination. Several procedures have been used to determine the hemoglobin concentration in catfish blood (Larsen and Snieszko, 1961b; and Larsen, 1964). The cyanmethemoglobin method has been found to be constant and the first choice for the use on catfish blood. A sample of 0.02 ml blood was mixed with 5 ml of Drabkins solution (Wintrobe, 1968). The amount of hemoglobin was then determined spectrophotometrically (at 540 mu) against a commercially prepared standard solution of cyanmethemoglobin (Ortho Diagnostics, Raritan, New Jersey). The hemoglobin is reported as gm/100 ml whole blood relative to man. Using this technique, the assumption was made that fish hemoglobin undergoes the same reactions with potassium-ferricyanide solution as does mammalian hemoglobin (Larsen and Snieszko, 1961b).

Absolute indices. The absolute indices were calculated for the brown bullhead catfish as follows: MCV (Mean Corpuscular Volume) in

$$\text{cubic microns} = \frac{\text{Hematocrit (\%)} \times 10}{\text{RBC } (10^6/\text{cu mm})};$$ MCH (Mean Corpuscular Hemoglobin) in pico-

$$\text{grams} = \frac{\text{Hemoglobin (gm \%)} \times 10}{\text{RBC } (10^6/\text{cu mm})};$$ MCHC (Mean Corpuscular Hemoglobin Concentration) in percent Weight/Volume $= \dfrac{\text{MCH}}{\text{MCV}}.$

Reticulocyte values. A sample of blood was drawn into a plain capillary tube. An equal volume of a one percent Brilliant Cresyl Blue solution in Locke's isotonic saline was drawn into the tube. The mixture was expelled and thoroughly mixed on a piece of parafilm, then taken back into the capillary tube for about five minutes, mixed again and a small drop was then

placed on a methanol cleaned slide to be smeared. After smearing, the slide was air-dried, fixed with absolute methyl alcohol and counterstained with Wright's stain (Humason, 1967). One thousand erythroid cells were counted per slide under oil immersion with the aid of a reticule and reported as per cent of reticulocytes.

Blood cell morphology. A drop of peripheral blood mixed with 3.8 percent sodium citrate was smeared on slides previously cleaned with 50 percent methanol, air-dried and fixed with absolute methanol. The blood smear preparations were then stained with Romanowsky stains: (1) Benzidine stain, Wright's, and Giemsa; (2) Wright's and Giemsa; and (3) May-Grünwald and Giemsa. The smears were examined with an oil immersion objective under 1000 X magnification, and measurements were made with an ocular micrometer.

All procedures were carried out under sterile conditions.

RESULTS

Blood Parameters

In an attempt to determine the normal hematological picture of the brown bullhead, seven fish were mechanically tagged by caudal fin clipping, transferred to a 50-gallon tank, and bled 0.25 ml at two week intervals, thereby allowing a comparison of the blood parameters of individual fish. Similarly, 25 fish were selected at random from a stock normal population, measured, and bled 0.25 ml. The results of the blood analyses and calculations for both groups of fish are listed in Table 1, which shows the total red cell numbers (RBC), total white cell numbers (WBC), reticulocytes (percent per 100 erythroid cells), hematocrit values, hemoglobin concentrations, Mean Corpuscular Volume (MCV, cu μ), Mean Corpuscular Hemoglobin (MCH, picograms, pg), and Mean Corpuscular Hemoglobin Concentration (MCHC, percent). The values are given as the mean, plus or minus one standard error of the mean; the number of fish used in each group is given in parenthesis.

Blood Cell Morphology

Compared to the cells of the erythrocytic series, the identification of the leucocytic series in fish is difficult, especially when attempts are made to discriminate between the granulocyte and agranulocyte. Furthermore, it is even more difficult to distinguish the young granulocytes and agranulocytes from the cells of the thrombocytic group.

The reported descriptions of the agranulocytes in fish are in disagreement, Yuki (1957, 1958) has attempted to resolve the discrepancy in classification of the young and transitional cells in the monocytic and granulocytic groups

in rainbow trout.

The difficulty in the task of correctly distinguishing thrombocytes and small lymphocytes lies in the fact (Saunders, 1968b) that some fish species have only mature thrombocytes in their peripheral circulation, while in others both mature and transitional cells can be found.

The following blood cells were detected in the peripheral blood of the brown bullhead: basophilic erythrocytes, reticulocytes, mature erythrocytes, senile or senescent erythrocytes, "nuclear shadows" or basket cells, round lymphocytes, elongated thrombocytes, round thrombocytes, fusiform or spindle-shaped thrombocytes, monocytes, neutrophils, eosinophils, macrophages, and hemocytoblasts. The enucleate erythrocyte or erythroplastid, which arises from the pinching off of a cytoplasmic portion of an erythrocyte, was occasionally found in the peripheral smear preparations but was not considered to be indicative of a blood dysfunction. No basophils were seen in any of the blood smear preparations examined.

The terminology of the cellular components of the brown bullhead used in this paper coincides with those proposed by Jakowska (1956); Chlebeck and Phillips (1969); Yuki (1960, 1963); Srivastava (1968); and Saunders (1967).

The erythrocytic series. The basophilic erythrocyte is slightly oval in shape, containing a centrally located, round nucleus. The size and shape of the basophilic erythrocyte varies, depending on the stage of polychromasia. The cytoplasm assumes a bluish-gray color and the nuclear fine chromatin architecture takes on a light purple color with both benzidine and Romanowsky staining. The cell size measures in the range of $9\mu \times 6\mu$ to $11\mu \times 8\mu$, and the nucleus is 3μ to 4μ in diameter. As with the basophilic erythrocyte, the slightly oval-shaped reticulocyte also varies in size, measuring in the range of $6\mu \times 5\mu$ to $10\mu \times 8\mu$, and its centrally located round nucleus measures 3μ to 4μ in diameter. The fine network of reticulum is clearly seen in a homogeneous pink cytoplasm, if the blood is mixed with a one percent solution of Brilliant Cresyl Blue before staining with Wright's stain.

The circulating normal mature erythrocyte has smooth margins and is predominantly ellipsoidal to oblong in shape and contains a centrally located round nucleus, 2μ to 5μ in diameter. The round erythrocyte measures from 8μ to 10μ in diameter and the ellipsoidal to oblong cell measures $11\mu \times 7\mu$ to $13\mu \times 10\mu$. The homogeneous cytoplasm of this mature cell takes on a pale green color and the nuclear thick chromatin-interchromatin network assumes a dark blue to violet color with benzidine and Romanowsky staining.

Senile or senescent erythrocytes are characterized by a loss of the smooth intact cell membrane and distended cytoplasm, which gives the cell its variability in size and shape. The cell measures $12\mu \times 10\mu$ with an eccentrically located variably-shaped nucleus, 5μ to 7μ in length. In the most advanced stage of degeneration, the nuclear membrane is no longer intact and its inner contents extend into the distended cytoplasmic portion of the cell. The cytoplasm takes on a pale green color and the nuclear clumped chromatin appears light blue or light purple when stained with benzidine and Wright's stains.

In the disintegrated erythrocytes ("nuclear shadows" or basket cells), the cytoplasm is no longer detected, and the cell therefore assumes a pale pink color when stained with benzidine and Romanowsky stains. An increase in the number of these cells may be due to either mechanical disruption during smear preparation or an increase in the fragility of erythrocytes which may be indicative of the numbers of senescent erythrocytes in the circulating blood. In some of the smear preparations highly refractile red serum granules, 1μ in diameter were found either within the cytoplasm or along the cell membrane of the erythrocyte. Highly refractile vacuoles larger than 1μ in diameter were also observed in cells of the same smear preparations. This infrequent occurrence of cytoplasmic inclusions may have been a result of the smear preparation technique.

Hemocytoblast or Hemoblast. In the circulation, this precursor cell measures from 8μ to 12μ in diameter. The centrally located large nucleus, 7μ to 8μ in diameter, with magenta-stained chromatin filaments and nucleoli, comprises almost the entire volume of the cell. The cytoplasm stains a deep blue with a Romanowsky stain. The hemocytoblast cell is the only direct derivative of the mesenchyme cell and differentiates into the leukogenic and erythrogenic cell series.

Lymphocytes. The round lymphocyte varies in size from 5μ to 7μ in diameter. Its round deeply basophilic nucleus with condensed chromatin comprises the entire volume of the cell. When treated with Romanowsky stains, the deep blue-purple to violet colored nucleus appears to be surrounded by a thin rim of a light pale blue cytoplasm. The irregular cellular outlines of cytoplasmic pseudopods characteristic of these cells are indicative of the lymphocyte's relatively rapid locomotion in the circulation. Vacuoles were not observed in these cells, however, in some instances red-colored azurophilic granules, about 1μ in diameter, approximately 20 per cell, were observed in the cytoplasm.

Thrombocytes. The brown bullhead contains elongated, round, and spindle or fusiform-shaped thrombocytes, some with pointed cytoplasmic terminal processes at one or both ends of the cell. The nucleus is centrally located and varies in outline according to the shape of the entire cell. The dimensions of the cell vary for each type: elongated cell—$10\mu \times 4\mu$ to $14\mu \times 5\mu$, nucleus $6\mu \times 3\mu$ to $9\mu \times 6\mu$; round cell—3μ to 4μ in diameter, nucleus 3μ in diameter; spindle or fusiform-shaped cell—$5\mu \times 3\mu$ to $9\mu \times 3\mu$, nucleus $4\mu \times 3\mu$. The very deep magenta to purple-stained compact nuclear chromatin is characteristic of the round and spindle shaped thrombocyte. In the elongated thrombocyte, the nuclear fine chromatin-interchromatin network takes on a magenta to purple color with Romanowsky stains. The nucleus of the thrombocyte in each of the above mentioned cells is surrounded by a homogeneous very pale blue to colorless cytoplasm, indicating the absence of basophilia. No granules or vacuoles were observed in the cytoplasm in any of the smear preparations, and nuclear indentations of the mature thrombocyte were not a frequent occurrence. Based on the fine network of nuclear chromatin and the slightly deeper blue cytoplasmic color, however, contrary to other investigators (Andrew, 1965), it is our belief that, in the brown bullhead, the elongated cell is the immature thrombocyte.

Granulocytes. The neutrophil is the predominant granulocyte in the circulating blood of the bullhead. It possesses an abundant clear pale blue to colorless cytoplasm surrounding an eccentrically located polymorphic magenta-stained nucleus. The mature neutrophil ranges from 10μ to 17μ in length, with a nucleus measuring from 4μ to 10μ in length. The loosely woven threadlike chromatin pattern of the nucleus may be round, kidney-shaped, ribbon-like, or more or less segmented in shape.

Eosinophils do not appear to be a frequent occurrence in the circulation of all brown bullheads. These large granulocytes, 10μ to 15μ in length, appear to be round to oval in shape. The relatively small, eccentric magenta-stained nucleus with clumped chromatin measures from 4μ to 6μ in length. The characteristic highly refractile eosinophilic red-orange granules, smaller than 1μ in diameter, averaging about 25 per cell, are dispersed throughout the colorless cytoplasm.

Monocytes. Monocytes in the brown bullhead vary in shape and measure 7μ to 14μ in length. The magenta-colored eccentrically-located nucleus is polymorphic in nature, ranging in shape from round, kidney, or bilobed and in size from 4μ to 8μ in length. The abundant cytoplasm of this mature circulating cell takes on a dull gray-blue color with Romanowsky stains. In a few

smear preparations, azurophilic granules, smaller than 1μ in diameter, appear in the cytoplasm. The occurrence of vacuoles in the cytoplasm is also infrequent.

Macrophages. The macrophage in this species of catfish is a very large highly vacuolated cell, frequently containing cellular debris. The appearance of the nucleus depends on the age of the cell. The very old cell contains a small distinct mass of magenta-stained clumped chromatin. In the younger cells, measuring 27μ to 31μ in length, the variably shaped nucleus is 6μ to 10μ in length. The cytoplasm in Romanowsky-stained blood smears takes on a dull gray color.

Discussion

The erythrocyte count, hematocrit, and hemoglobin values of the brown bullhead reported here compare favorably with those reported by Haws and Goodnight (1962), Table 2. Although there were no significant differences in the hematocrit or hemoglobin concentration values, statistical analyses indicate a significant difference in red blood cell count, white blood cell count, reticulocyte count at 17 days after bleeding the seven individual fish ($P \leqq 0.05$). There is also a trend of an increase in the MCV which is to be expected if there is a depletion of the reticulocyte compartment resulting in a red blood cell population consisting predominantly of mature erythrocytes.

The differences observed in the reported values cannot be attributed to any change in the temperature, oxygen level, or pH of the experimental tank, since these values did not alter significantly during the time interval in which the seven tagged fish were housed.

The hematological values obtained for the group of randomly selected 25 fish are given in Table 1. The most striking difference is observed in the total white blood cell values. This difference may be accounted for by the stressed condition to which these fish were subjected when the fish were individually caught for blood sampling. In fact, the differential counts of the individual fish of this group show a definite tendency towards a decrease in lymphocyte count and an increase in thrombocyte count as the fish were subjected to stress for a longer period of time (Table 3). Consequently, the mean differential values for the group of seven fish differ significantly from those for the group of 25 fish. Otherwise, the differential counts of both groups are comparable (Table 3).

Studies with the killifish conducted by Pickford, et al. (1971a, 1971b, 1971c) have shown a definite correlation between stress and changes in the abundance of circulating leukocytes, as shown by alternating sequences of leukopenia and leukocytosis. The typical sequence of recovery was described as follows: leukopenia at three min, leukocytosis at 15 min, leukopenia at 30 to 60 min, leukocytosis at 2 hrs, followed by a gradual return to normal.

The only other differential analysis reported for catfish blood has been for the channel catfish species, *Ictalurus punctatus* (Dodgen and Sullivan, 1969). Their findings are comparable with those reported in Table 3, in that the predominant cell found in the peripheral circulation is the lymphocyte.

Differential counts for other species of fish have also reported the lymphocyte as the prevailing white blood cell form, e.g., pike (Mulcahy, 1970), goldfish (Watson and Shechmeister, 1963; Weinreb, 1963), and killifish (Pickford, et al. 1971a). This is in contrast to studies done by Saunders with 121 species of

TABLE 1. THE HEMATOLOGICAL PARAMETERS OF THE BROWN BULLHEAD CATFISH, *Ictalurus nebulosus* (LE SUEUR).

The values are given as the mean, plus or minus one standard error of the mean. The number of fish used in each group is shown in parenthesis.

	RBC 10^6/cu mm	WBC 10^3/cu mm	Retics %	Hct %	Hb gm/100 ml	MCV cu μ	MCH pg	MCHC %
Group I (7)								
T₀	1.79 ±0.067	94.7 ± 3.6	13.0 ± 0.70	24.2 ± 0.59	8.14 ±0.39	136.0 ± 3.60	45.9 ± 3.3	33.1 ± 1.9
T 17 days*	1.47 ±0.116	71.9 ± 6.4	7.13 ± 1.19	23.2 ± 0.76	7.84 ±0.31	160.8 ± 8.11	54.2 ± 2.6	33.1 ± 1.0
T 27 days**	1.44 ±0.056	65.6 ± 8.9	4.92 ± 1.00	24.7 ± 0.93	6.42 ±0.21	172.2 ± 5.88	44.7 ± 1.8	25.7 ± 0.60
Group II (25)	1.69 ±0.051	46.2 ± 2.8	8.79 ± 1.02	30.2 ± 1.00	8.80 ±0.24	180.2 ± 5.18	53.1 ± 1.3	29.0 ± 0.34

* 17 days after first bleeding of 0.25 ml per fish.

** 27 days after first bleeding of 0.25 ml and 10 days after second bleeding of 0.25 ml.

marine fish of Puerto Rico (1966a), 50 species of fish from the Red Sea (1968a), several species of elasmobranchs (1966b), and Gardner and Yevich (1969) with cyprinodontiform species; here the thrombocyte was found to be the predominant cell in differential counts of blood smear preparations.

Summary

A study was made of the hematological parameters and blood cell morphology of a normal population of brown bullhead catfish presented with the intent that these would serve as baseline figures for comparison with values obtained from fish that had been subjected to various forms of hypoxia and other stresses.

The changes in hematological values obtained

as a result of blood letting indicated that recovery from an induced state of anemia did not occur before 17 days post bleeding.

Further research is being conducted on the hematological parameters and blood cell morphology of other species of fish, and the physiological control of blood cell formation in different species of fish is presently being examined.

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Table 2. Comparison of present results with those of Haws, *et al.* in the brown bullhead catfish.

	Mean	(Range)
Erythrocyte counts: 10^6 cells/cu mm		
Weinberg, *et al.*	1.69	(0.144– 1.74)
Haws, *et al.*	1.22	(0.75 – 1.94)
Hematocrit (%)		
Weinberg, *et al.*	27.2	(23.0 –31.2)
Haws, *et al.*	27.9	(15.0 –47.0)
Hb in gm/100 ml		
Weinberg, *et al.*	8.8	(7.6 –10.0)
Haws, *et al.*	6.9	(4.0 –10.0)
Erythrocyte length (μ)		
Weinberg, *et al.*	—	(9.0 –13.0)
Haws, *et al.*	—	(11.4 –15.9)
Erythrocyte width (μ)		
Weinberg, *et al.*	—	(7.0 –10.0)
Haws, *et al.*	—	(7.6 –11.4)

Table 3. Differential Counts of the Brown Bullhead Catfish.

The values are given as the mean, plus or minus one standard error of the mean. The number of fish used in each group is shown in parenthesis.

	LYMPHO-CYTES %	THROMBO-CYTES %	NEUTRO-PHILS %	MONO-CYTES %	MACRO-PHAGES %	HEMOCY-TOBLASTS %	EOSINO-PHILS %
Group I (7)							
T₀	67.4±4.5	23.6±3.0	6.9±2.5	1.3±0.3	0.08±0.05	0.58±0.30	0.00
T 17 days*	67.9±3.6	22.1±4.7	6.9±3.1	1.7±0.9	0.15±0.10	0.90±0.36	0.00
T 27 days**	66.5±6.2	24.8±6.1	6.1±1.9	1.0±0.3	0.98±0.84	0.45±0.18	0.00
Group II (25)	30.71±2.3	58.6±2.3	7.2±1.1	1.7±0.2	0.82±0.27	0.19±0.05	0.18±0.11

* 17 days after first bleeding of 0.25 ml per fish.

** 27 days after first bleeding of 0.25 ml and 10 days after second bleeding of 0.25 ml.

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4

Histochemical Analyses of the Fluid and the Solid State of the Adhesive Materials Produced by the Pre- and Postmetamorphosed Cyprids of *Balanus eburneus* Gould

(Figures 1-6; Tables 1-10)

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Two distinct eosinophilic zones of granules found in the larval (cyprid) cement gland of *Balanus eburneus* Gould are referred to as medial and inner granules, according to their morphological position within the gland. No such differentiation of the secretory materials are present in the adult cement cells. Histochemical tests show that the granules, which represent the fluid state of the cement material, in the cyprid and adult, are basic protein, characterized as collagenous substances. The *hardened* cement produced by the cyprid and the adult that is found on the basal plate are histochemically unreactive protein masses, but the two substances are not histochemically identical.

Introduction

In recent years, studies on barnacles have been concerned with the cement, its synthesis, and the cement-producing complex. Thus, the general morphology of the cement gland in the cyprid stage was reported by Bernard and Lane (1962), the histology by Walley (1969), and the ultrastructure of the gland and the attachment pads of the antennules by Nott (1970), Nott and Foster (1970), and more recently by Walker (1971).

The histology of the cement apparatus of several species of adult barnacles was described in detail by Lacombe (1966, 1967, 1970), Lacombe and Liguori (1969), and at the ultrastructural level, by Walker (1970).

Costlow (1959) demonstrated the presence of carbonic anhydrase in the shell-forming tissue of the adult barnacle. Arvy and Lacombe (1968), Arvy, Lacombe, and Shimony (1968), and Shimony and Nigrelli (1971a, b) reported the presence of succinic dehydrogenase, alkaline phosphotase, arylsulphatase, and polyphenoloxidase, respectively, in the cement and cement apparatus of the adult barnacle, while Walker (1971) demonstrated polyphenoloxidase in both the gland and in the secreted cement of the cyprid stage of *Balanus balanoides*.

On the basis of histochemical techniques, various opinions were presented as to the chemical nature of the barnacle cement. Hillman and

Nace (1970) and Shimoy (1971) concluded that the cement laid down by the attached cyprids was collagen, an opinion with which we concur in this report. Walker (1970, 1971) and Saroyan, *et al* (1970a) stated that the cement substances of the cyprid and the adult were proteins with phenolic groups, while Lacombe (1968) concluded that the cement in the adult barnacle consisted of acid mucopolysaccharides.

These reports show the confusion in defining the chemical nature of the cement at a particular stage. The present paper deals with histochemical analyses of the fluid and solid states of the adhesive materials produced by the pre- and post-metamorphosed cyprids of *Balanus eburneus* Gould.

Materials and Methods

Adult barnacles were collected locally or obtained from Biscayne Bay (Florida), attached to aluminum plates, and air shipped the same day to New York. The cyprids were raised in the laboratory and allowed to metamorphose to the barnacle stage in beakers. The glass around each barnacle was then cut in such a way that it served as a slide for microscopical examinations following treatment for various histochemical reactions.

Cyprids and barnacles were fixed in 10% buffered formalin, Bouin's fluid, Heidenhain's Susa, Zenker's, and Carnoy's solutions for general histological and histochemical observations. Where necessary, 5% nitric acid was used for

decalcification. After fixation and decalcification the animals were embedded in paraplast and sectioned at 6 μ. The various histochemical methods employed in this study are summarized in Tables 1-5.

TABLE 1. GENERAL HISTOLOGICAL AND HISTOCHEMICAL METHODS USED TO DEMONSTRATE VARIOUS COMPONENTS OF THE CEMENT APPARATUS OF THE BARNACLE, *Balanus eburneus* GOULD

Methods	Fixatives	References	Purposes
Hematoxylin and eosin	10% neutral formalin	Thompson (1966)	General structures; stain for acidic and basic components of the tissue
Azure A and eosin B pH 3.7–4.3	10% neutral formalin	Conn *et al* (1960)	General structures
Mallory's phloxine methylene blue	10% neutral formalin	Mallory (1938)	Stain for collagen
Masson's Trichromes	10% neutral formalin	Masson (1929)	Stain for collagen and intracellular fibers
Gomori's Trichromes	10% neutral formalin	Gomori (1950)	Stain for collagen and connective tissues
Phosphotungstic acid hematoxylin	10% neutral formalin and Susa	Lillie (1954)	Stain for collagen
Mallory's collagen stain aniline blue and orange G	Zenker's fluid	Mallory (1936)	Stain for collagen

TABLE 2. METHODS EMPLOYED TO DEMONSTRATE CARBOHYDRATE COMPONENTS AND METACHROMASIA OF THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Periodic Acid Schiff (PAS)	Susa and 10% neutral formalin	Thompson (1966)	Demonstration of adjacent glycol or amino-hydroxy groupings
PAS + Acetylation	10% neutral formalin	Lillie (1954)	Blocking 1,2-glycols and 1,2-aminohydroxy groups in oxidative Schiff reaction
PAS + Saponification	10% neutral formalin	Lillie (1954)	Deacetylation
PAS + Saliva digestion	10% neutral formalin	Lillie (1949)	Removal of glycogen and RNA
Mucicarmine	10% neutral formalin and Carnoy's fluid	AFIP (1957)	Demonstration of acid mucopolysaccharide (mucin) of epithelial origin
Bast's Carmine	Susa and Carnoy's fluid	Thompson (1966)	Demonstration of glycogen
Azure A 0.1% and 0.01% pH 3.9	Susa and Carnoy's fluid	Thompson (1966)	Demonstration of metachromasia
Methenamine silver nitrate	10% neutral formalin	Gomori (1946)	Demonstration of glycogen and mucin
Alcian blue pH 2.8	Susa and Bouin's fluid	Thompson (1966)	Demonstration of sulfated acid mucopolysaccharide
Ribonuclease + Alcian blue pH 2.8	Susa and Bouin's fluid	Thompson (1966)	Hydrolysis of RNA
Alcian blue pH 2.8 + sulfation	Susa and Bouin's fluid	Thompson (1966)	Demonstration of metachromasia by esterification of carbohydrates
Toluidine blue 0, at 0.01% pH 2.8 + sulfation	Susa and Bouin's fluid	Thompson (1966)	Demonstration of metachromasia by esterification of carbohydrates

TABLE 3. METHODS EMPLOYED TO DEMONSTRATE NUCLEIC ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Methylene blue pH 3.0	Susa's fluid	Stenram (1953)	Demonstration of nucleic acids; RNA and DNA
Ribonuclease + Methylene pH 3.0	Susa's fluid	Stenram (1953)	Enzymatic hydrolysis of RNA
Toluidine blue 0, 0.5% pH 3.0	Susa's fluid	Stenram (1953)	Demonstration of nucleic acids; RNA and DNA
Ribonuclease + Toluidine blue 0, 0.5%, pH 3.0	Susa's fluid	Stenram (1953)	Enzymatic hydrolysis of RNA
Nucleal Feulgen Reaction	Susa's fluid	Thompson (1966)	Demonstration of DNA

TABLE 4. METHODS EMPLOYED TO DEMONSTRATE LIPIDS AND UNSATURATED FATS IN THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Sudan black B	Unfixed tissue and 10% neutral formalin	Thompson (1966)	Demonstration of liquid, and semi-solid fats in tissues
Sudan black B + acetone	Unfixed tissue and 10% neutral formalin	Thompson (1966)	As control
Plasmal Reaction	10% neutral formalin	Hayes (1949)	Demonstration of unsaturated fats
Luxol fast blue	10% neutral formalin	Thompson (1966)	Demonstration of phospholipids

TABLE 5. METHODS EMPLOYED TO DEMONSTRATE PROTEIN AND AMINO ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Fixatives	References	Purposes
Tetrazotized benzidine with β-naphthol	Bouin's Fluid 10% formalin	Lillie (1957)	Demonstration of proteins in general
Ninhydrin Schiff	Susa's fluid	Yasuma et al (1953)	Demonstration of the sites of free α-amino acids
2,2'-dihydroxy-6, 6'-dinaphthyl	Susa's fluid 10% formalin	Thompson (1966)	Demonstration of the sulfhydryl group and
disulfide and thioglycolic acid (DDD)	Carnoy's fluid		disulfide linkages
DDD without thioglycolic acid	Susa's fluid	Thompson (1966)	Demonstration of the sulfhydryl group
DDD with benzoyl chloride	Susa's fluid 10% formalin	Thompson (1966)	Demonstration of the disulfide linkages
Mercury Orange	Susa's fluid Carnoy's fluid	Thompson (1966)	Demonstration of the sulfhydryl group
8-hydroxyquinoline	Susa's fluid	Lillie (1957)	Demonstration of amino acid arginine
p-dimethylaminobenz-aldehyde nitrite	Susa's fluid 10% formalin	Adams (1957)	Demonstration of indole derivative (tryptophan)
Diazotization with 8-amino-1-naphthol-5-sulfonic acid	10% formalin	Glenner and Lillie (1959)	Demonstration of tyrosine and phenolic compounds
Millon's Reagent	10% formalin	Thompson (1966)	Localization of tyrosyl groups in tissue sections

DESCRIPTION OF THE CEMENT APPARATUS[1]

The Cyprid Cement Gland

The cement glands in the cyprid of *Balanus eburneous,* as in the other species of the Balanidae, are paired kidney-shaped structures with an average measurement of $70 \times 50 \times 70$ microns. Each gland consists of a number of secretory cells arranged as compartments. Each secretory cell (Figure 1) contains a large amount of two histochemically distinct secretory granules and a peripherally located nucleus. These granules referred here as medial and inner granules according to their relative position within the cement gland of the cyprid. The α- and β-cells reported by Walker (1971) are not easily discerned with the light microscope. However, the medial and the inner granules are probably the two types of electron-dense bodies found in the α-cells. The collecting canal arises within the medullary portion of the gland and then passes into the conducting canal through the antennule to the attachment pad (Figure 2).

The Adult Cement Apparatus

The cement secreting cells in the adult barnacle are not enclosed in a compact glandular structure; instead the cells are scattered in the mantle tissue mainly along the lateral axis with the associated canals. The cement cells are large, up to 140 microns in diameter, with unusually large polymorphic nuclei containing clumps of chromatin material. The cytoplasm has evenly distributed granules and densely staining secretory zones. No vacuoles were noted at the secretory zones (Figure 3). The smallest cement cell with secretary zones measures 14 microns in diameter; the circular nucleus contains a centrally located nucleolus. The collecting canal makes its contact with the cement cell at secretory zone (Figure 4), and the conducting canal links the individual cement cells into grape-like clusters. The intracellular cement substance accumulated at the secretory zones is delivered to the exterior through the canal system in the basal plate.

The Basal Plate Structures

The basal plate of *Balanus eburneus* Gould is a complicated structure. At the center of the basal plate, the region of the initial point of attachment of the cyprid, the hardened cement consists of two spots approximately 18 microns in diameter, with the remains of the segment IV and the attachment pads of the antennules attached to the substrate. The initial cement of the adult barnacle, located anteriorly to the cyprid cement, also appears as two spots, approximately 35 microns in diameter, with the broken portion of the conducting canal remaining at the

center of each spot (Figure 5). Two radial canals are formed by branching along the lateral axis from the conducting canals at the center of the base with circular canals growing out from the radial canals. The adhesive substance is delivered to the edge of the growing plate through the openings in the circular canals. Figure 6 is a composite schematic representation of the cement apparatus of the pre- and post-metamorphosed cyprid at the region of initial attachment.

HISTOLOGICAL AND HISTOCHEMICAL REACTIONS

The results of the histological and histochemical reactions on various tissue and cellular components of the cement apparatus of the pre- and post-metamorphosed stages of the barnacle, *Balanus ebureus* Gould, are summarized in Tables 6 to 10.

The medial and the inner granules of the cyprid cement secreting cells show differences in (1) degree of acidophilia; (2) permeability to orange G and hematin-lake formation; and (3) intensity in reaction with carbohydrate and amino acid detecting agents.

The granules in both locations have similar histochemical reactions and stained positively with a number of collagen identifying agents. The medial and the inner granules are characterized by the presence of (1) tryptophan, arginine, and a number of α-amino acids; (2) small amount of lipid and phospholipid substance; (3) no unsaturated fats; (4) carbohydrate components of small molecular size; and (5) sulfhydryl group and disulfide linkage. These two types of granules probably represent stages in synthesis.

The cement material at the secretory zones of the adult cement cell does not differentiate into two acidophilic zones. This fluid cement is also identified as a collagenous substance. The carbohydrate reactions are less intense in comparing with the cyprid inner granules. A small amount of lipid and phospholipid substances are present. Neither unsaturated fats nor ribonucleic acid are found in the cement. No metachromasia is demonstrated. Tyrosine is found in the adult cement but absent in the cyprid of *Balanus eburneus.*

The hardened cyprid cement, i.e. the cyprid antennular deposits at the center of the basal plate, is not stained by the acid and basic dyes and is nearly histologically and histochemically inert. However, the free adjacent glycol or amino-hydroxy groupings can still be detected with PAS. Furthermore, the cement in the hardened state is apparently a protein mass but no specific amino acids could be demonstrated when treated by the usual chemical agents. This observation is in agreement with the histochemi-

[1] A glossary of terms used in this text is given in the appendix.

cal analyses of the cyprid hardened cement by Hillman and Nace (1970).

The hardened adult barnacle cement appears as rings associated with the circular canals on the basal plate, which becomes histochemically non-reactive but has the characteristic of a basic protein. It differs from the hardened cyprid cement by the following characteristics: (1) positive alkaline nature; (2) PAS negative; and (3) the presence of tyrosyl groups.
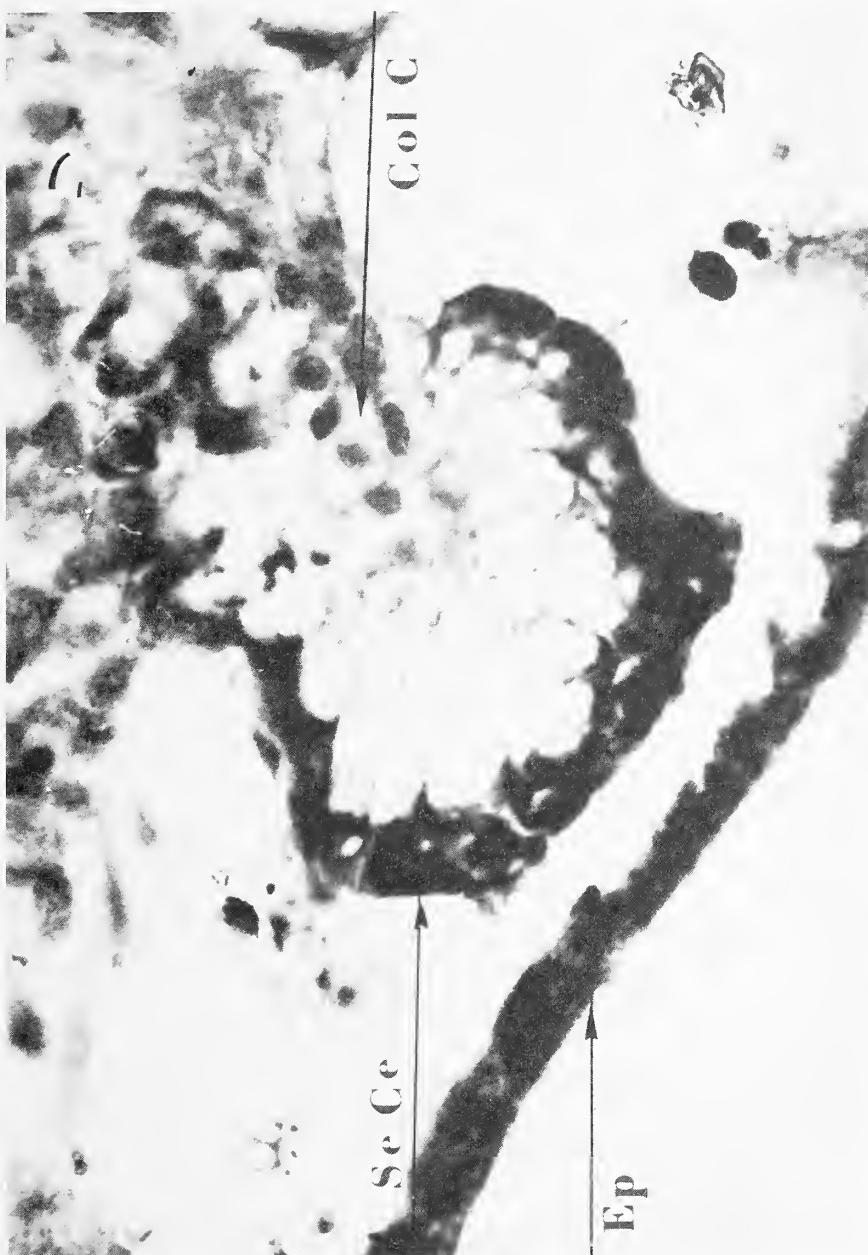
FIGURE 1. A section through the cyprid cement gland showing the cortically located secretory cells and the collecting canal at the medullary portion of the gland; the cytoplasmic granules are colorless with the tribastic stains. Col C, collecting canal; Se Ce, secretory cells; Ep, epithelial cells. (43 X).
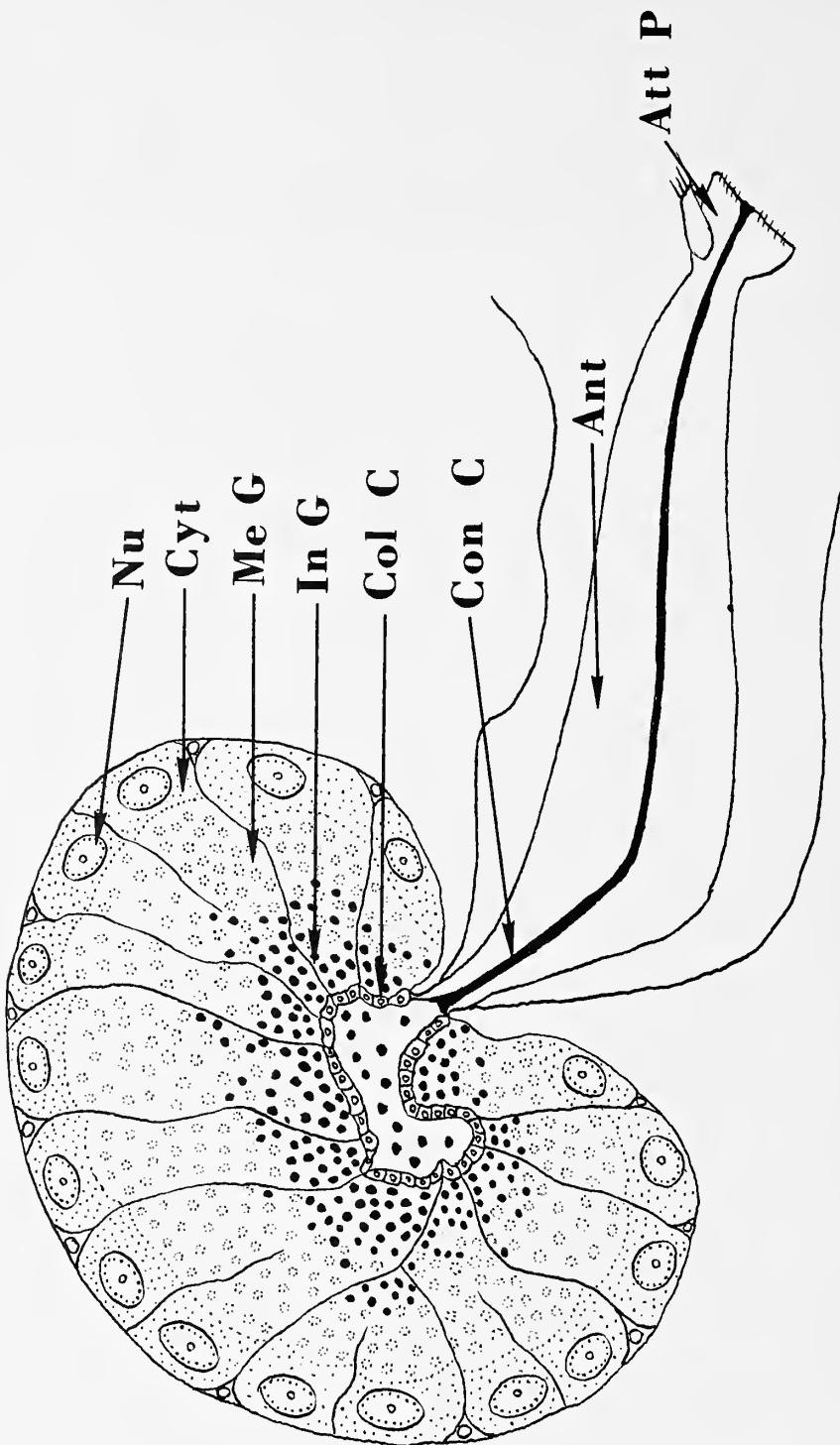
FIGURE 2. A composite schematic representation of the cyprid cement apparatus showing the relative position of the medial and the inner granules within the gland; Nu, nucleus of the secretory cell; Cyt, cytoplasm; Me G, medial granules; In G, inner granules; Col C, collecting canal; Con C, conducting canal; Ant, antennule; Att P, attachment pad.
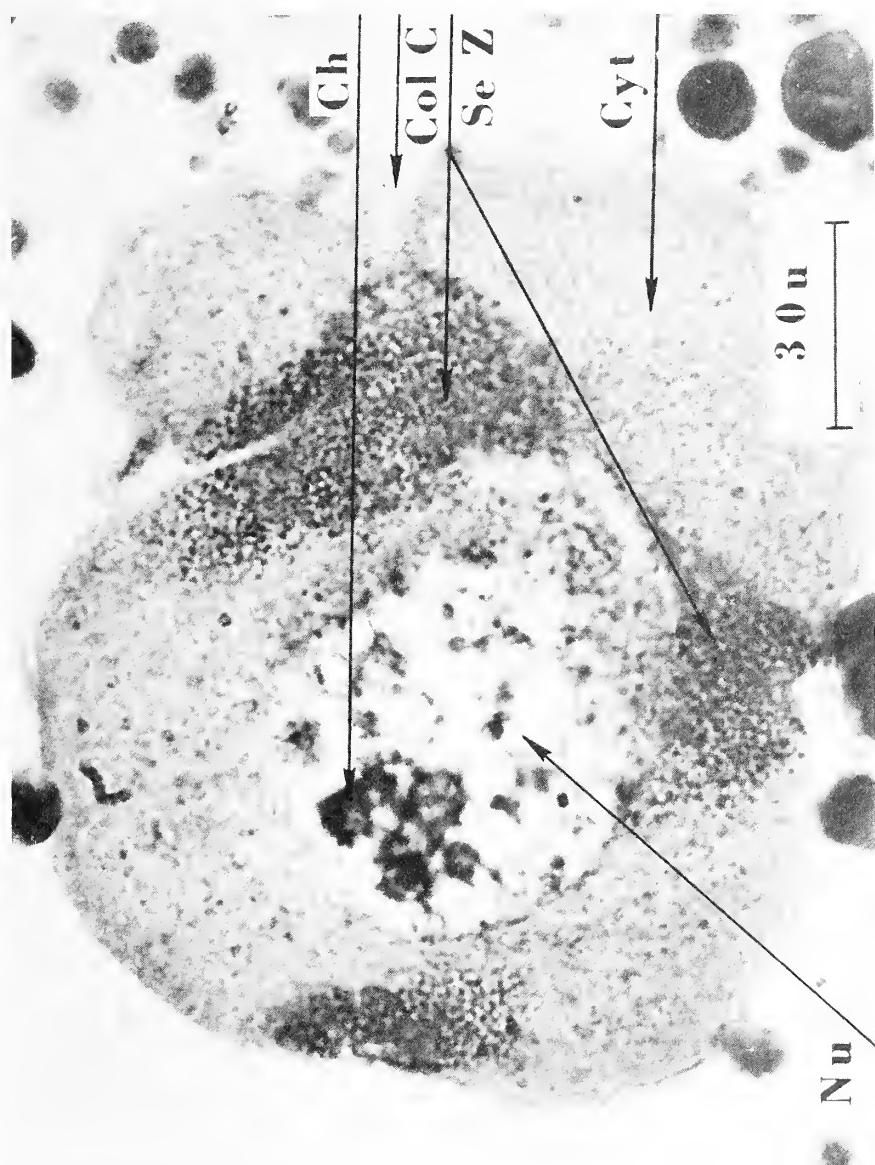
FIGURE 3. A section through an adult cement cell of *Balanus eburneus* Gould; the cytoplasm is grayish and the three secretory zones are pink with Mallory's phloxine stains; Ch, chromatin materials; Col C, collecting canal; Se Z, secretory zone; Cyt, cytoplasm; Nu, nucleus. (100 X).
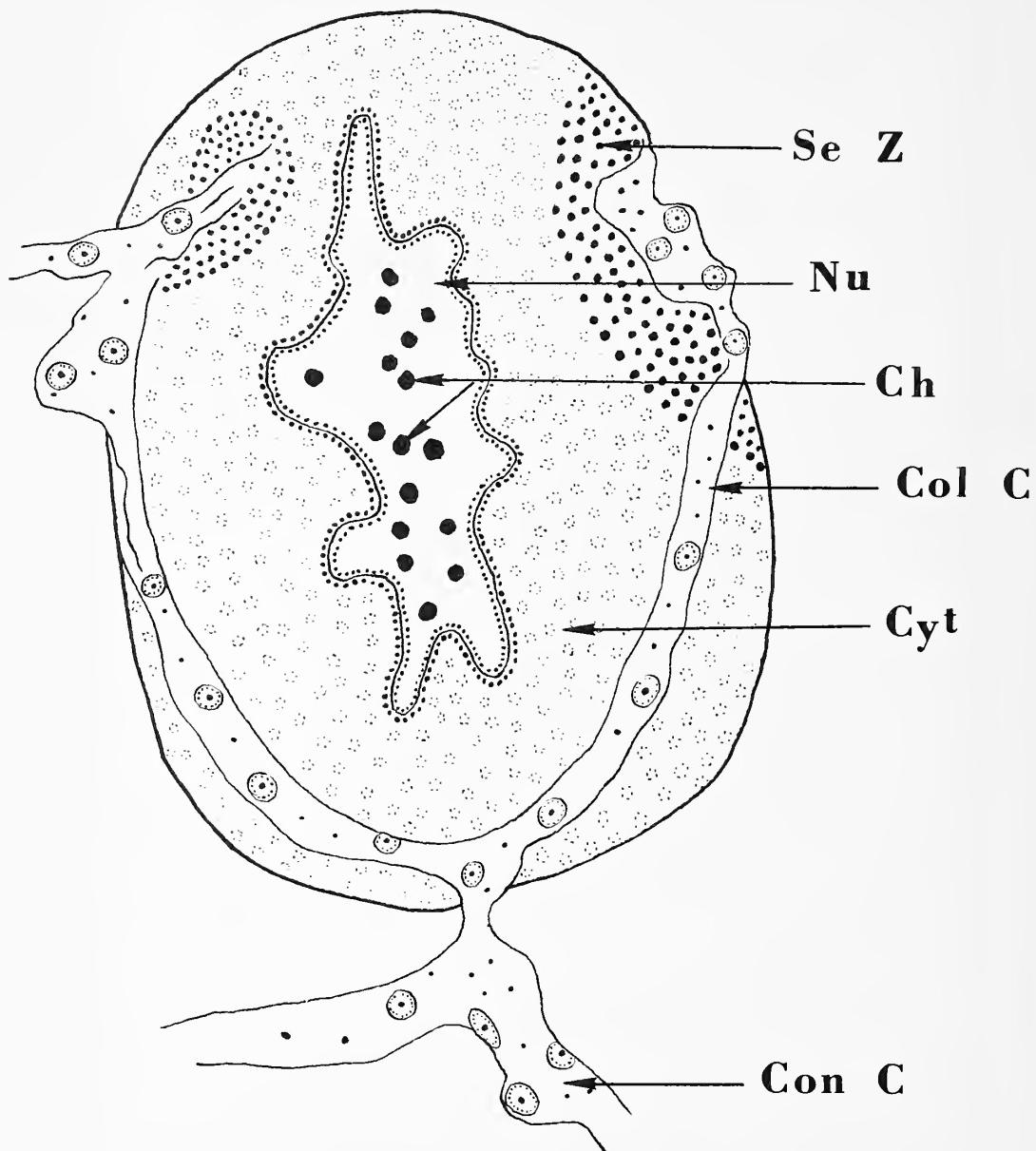
FIGURE 4. A schematic representation of the adult barnacle cement cell associated with the collecting and the conducting canals; Se Z, secretory zone; Nu, polymorphic nucleus; Ch, chromatin materials; Col C, collecting canal; Cyt, cytoplasm; Con C, conducting canal.
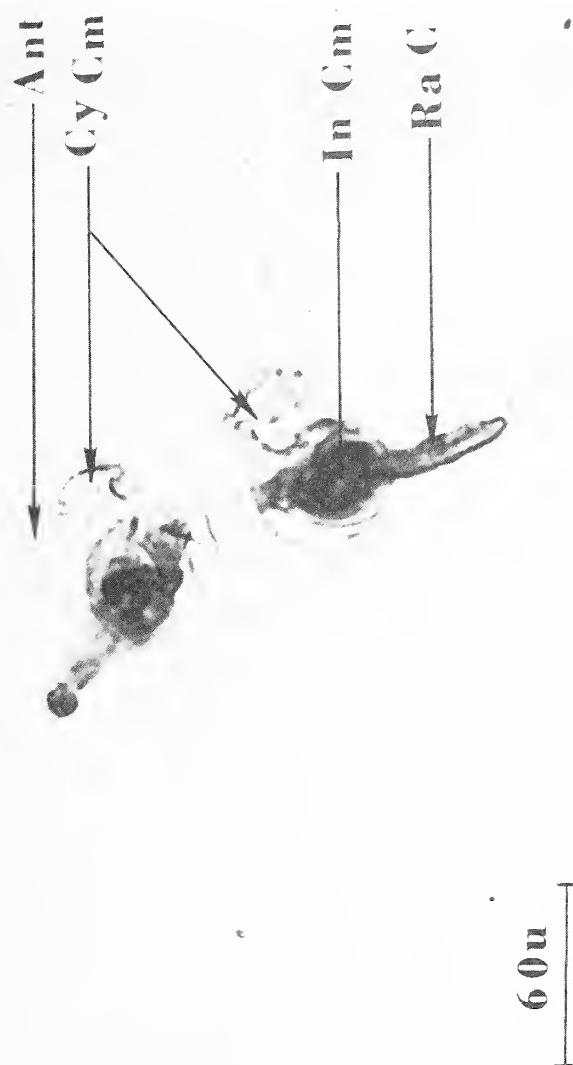
FIGURE 5. The area of initial attachment on the basal plate of *Balanus eburneus* Gould; the cyprid cement is colorless while the adult barnacle cement and the radial canal are pink when treated with Millon's Reagent; Ant, segment IV of the antennule; Cy Cm, cyprid ceme ntwith the antennular attachment pad; In Cm, initial cement of an adult barnacle; Ra C, radial canals branching from the center of the basal plate. (43 X).
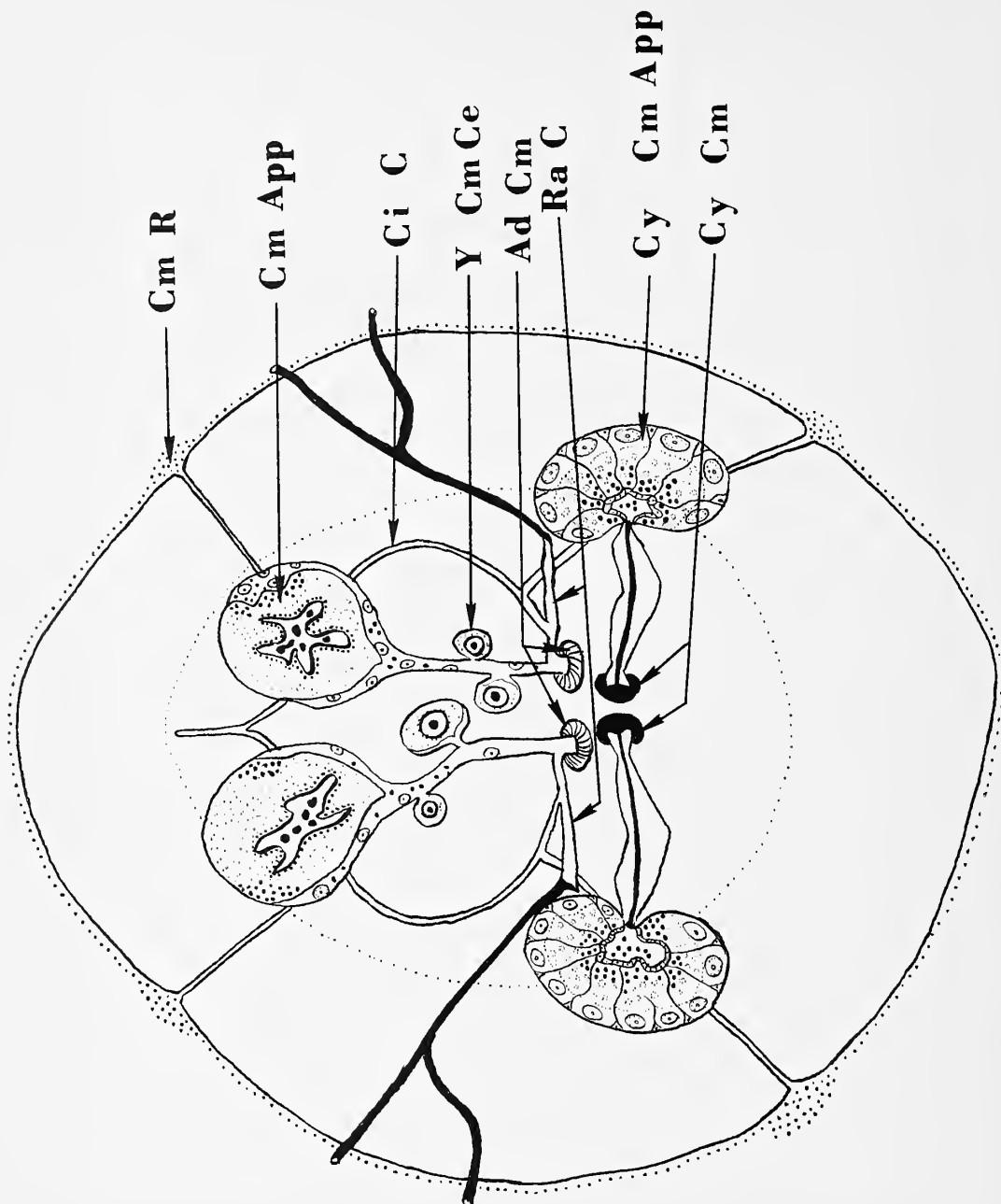
FIGURE 6. A composite schematic representation of the area of the initial attachment made by the barnacle showing the relationship between the cyprid cement gland and the adult cement apparatus; Cm R, cement ring; Cm App, cement apparatus; Ci C, circular canal; Y Cm Ce, young cement cell; Ad Cm, adult cement; Ra C, radial canal; Cy Cm App, cyprid cement apparatus; Cy Cm, cyprid cement.

TABLE 6. GENERAL HISTOLOGICAL AND HISTOCHEMICAL REACTIONS OF VARIOUS COMPONENTS OF THE CEMENT APPARATUS OF THE BARNACLE, *Balanus eburneus* GOULD

Methods	Adult Cement Cell		Cyprid Cement Cell			Antennule Deposit	Basal Plate	
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules		Fluid Cement	Hardened Cement
Hematoxylin and eosin	L. blue	pink	blue	purple	pink	pink	pink	pink
Azure A and eosin B	blue	pink	blue	pink	red	colorless	pink	pink
Mallory's phloxine and methylene blue	blue	red	blue	purple	red	colorless	red	red
Masson's Trichromes	violet	violet	red	blue	red	colorless	red	red
Gomori's Trichromes	violet	violet	red	L. blue	red	colorless	red	red
Phosphotungstic acid Hematoxylin	purple	L. brown	purple	L. brown	blue	colorless	purple	colorless
Mallory's collagen stain aniline blue and orange G	purple	orange	purple	blue	orange	colorless	orange	colorless

TABLE 7. HISTOCHEMICAL TESTS FOR CARBOHYDRATES IN THE BARNACLE ADHESIVES(S)

| | Structures | | | | | | | |
| | Adult Cement Cell | | Cyprid Cement Cell | | | | Basal Plate | |
Methods	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Periodic Acid Schiff (PAS)	L. pink	pink	pink	pink	pink	pink	pink	colorless
PAS + acetylation	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
PAS + Saponification	L. pink	pink	pink	L. pink	L. pink	pink	pink	colorless
PAS + Saliva digestion	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Mucicarmine	red	red	red	pink	red	colorless	colorless	colorless
Best's Carmine	pink	L. pink	red	pink	pink	.....	.....	.....
Azure A 0.1% and 0.01% at pH 3.9	blue	colorles	bule	colorless	colorless	colorless	colorless	colorless
Methenamine silver nitrate	L. black	black	colorless	L. black	black	colorless	colorless	colorless
Alcian blue pH 2.8	blue	blue	blue	blue	blue	colorless	colorless	colorless
Ribonuclease + Alcian blue pH 2.8	blue	blue	blue	blue	blue	colorless	colorless	colorless
Alcian blue + Sulfation	dissolved	dissolved	blue	blue	blue	L. blue	colorless	colorless
Toluidine blue 0 + Sulfation	dissolved	dissolved	blue	blue	blue	blue	colorless	colorless

TABLE 8. HISTOCHEMICAL TESTS FOR NUCLEIC ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Adult Cement Cell		Cyprid Cement Cell				Basal Plate	
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Methylene blue	blue	blue	blue	colorless	colorless	colorless	colorless	colorless
Ribonuclease + Methylene blue	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Toluidine blue 0	blue	colorless	blue	colorless	colorless	colorless	colorless	colorless
Ribonuclease + Toluidine blue 0	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Feulgen Reaction	colorless	colorless	colorless	colorless	colorless	......	......	......

TABLE 9. HISTOCHEMICAL TESTS FOR LIPIDS AND UNSATURATED FATS IN THE BARNACLE ADHESIVE(S)

Methods	Adult Cement Cell		Cyprid Cement Cell				Basal Plate	
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Sudan black B	colorless	blue	L. black	L. black	colorless	colorless	L. black	colorless
Sudan black B + Acetone	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Plasmal Reaction	L. pink	colorless	L. pink	colorless	colorless	colorless	colorless	colorless
Luxol fast blue	colorless	blue	colorless	colorless	blue	blue	blue	blue

TABLE 10. HISTOCHEMICAL TESTS FOR PROTEINS AND AMINO ACIDS IN THE BARNACLE ADHESIVE(S)

Methods	Adult Cement Cell		Cyprid Cement Cell				Basal Plate	
	Cytoplasm	Secretory Zone	Cytoplasm	Medial Granules	Inner Granules	Antennule Deposit	Fluid Cement	Hardened Cement
Tetrazotized benzidine with β-naphthol	orange	orange	orange	orange	orange	orange	orange	orange
Ninhydrin Schiff α-amino acids	pink	pink	pink	L. pink	pink	pink	pink	pink
2,2'-dihydroxy-6,6'-dinaphthyl disulfide (DDD) for SH- & S-S	brownish	purple	orange	L. orange	purple	colorless	L. purple	colorless
DDD without thioglycolic acid for SH- group	orange	brownish	orange	orange	orange	colorless	L. purple	colorless
DDD with benzoyl chloride for S-S	brownish	purple	orange	orange	purple	colorless	L. purple	colorless
Mercury Orange	orange	L. orange	orange	L. orange	orange	colorless	orange	colorless
8-hydroxyquinoline for arginine	orange	orange	orange	orange	orange	colorless	orange	colorless
p-dimethylamino-benzaldehyde nitrite for tryptophan	colorless	blue	colorless	blue	blue	colorless	blue	colorless
Diazotization with 8-amino-1-naphthol-5-sulfonic acid for tyrosine	L. orange	orange	colorless	colorless	colorless	colorless	violet	violet
Millon's Reagent for tyrosine	L. orange	orange	colorless	colorless	colorless	colorless	red	orange

DISCUSSION

. Histological observations on serial sections of a newly metamorphosed barnacle show that the adult cement apparatus is not derived from the remnant of the cyprid cement gland. Therefore, these two cement producing organs have different origins and developments. Based on the morphological differences of the cement apparatus, it is assumed that the adhesive substances produced by the pre- and post-metamorphosed cyprid are different.

Therefore, it should be emphasized that our histochemical studies deal with the following: (1) the intracellular fluid material of the cyprid cement gland; (2) the "hardened" cement at the points of antennular attachment of the cyprid; (3) the intracellular and intracanicular fluid cement of the post-metamorphosed barnacle; and (4) the "hardened" cement of the initial and subsequent deposits produced by the adult.

Further, it should be recognized that our studies deal mainly with the surface chemistry of the so-called "hardened" cement of the cyprid and adult, which may account for some of the differences in the histochemical reactions noted by us and those reported by other investigators (Walker 1970, 1971; Hillman and Nace, 1970; and Saroyan, *et al,* 1970a).

The histochemical analyses of the fluid and the solid state of the cement suggest that the materials are collagenous which undergo a transition from a highly chemically reactive (fluid cement) to an unreactive mass upon hardening.

The fluid cement of the cyprid is present in the treated material as medial and inner granules characterized histochemically by differences in the numbers of free cationic groups as reflected by differences in the degree of acidophilia of these two granules. It is our belief that these granules are the same as those reported by Walker (1971) as two types of electron-dense bodies within the α-cells of the cyprid cement gland. It further leads us to infer that the inner granules (i.e., more electron-dense bodies) are probably a polymerized state of the medial granules (i.e., less electron-dense bodies), which may represent the monomeric state of the adhesive. The above interpretations may also explain the difference in the intensity of most of the histochemical reactions of these two types of granules.

All of the histochemical tests, as mentioned above, indicate that the fluid cement of both the pre- and post-metamorphosed cyprids is a collagenous substance. The presence of sulfhydryl groups and disulfide linkage indicates that the structure of the cement has a compactly coiled and/or folded configuration.

The presence or absence of tyrosine in the cement material raises some questions. Our results showed that this amino acid is not present in the fluid and hardened cement of the cyprid (see also Hillman and Nace, 1970), but is found in the cement of the adult (Figure 5). This is contrary to that reported by Saroyan *et al* (1970a) and by Walker (1970, 1971) who demonstrated tyrosine in the cement of both the adult and cyprid. The significance of the lack of the demonstrable tyrosyl group in the cyprid cement is obscure.

The interpretation that the cement is an acid mucopolysaccharide, as has been suggested by several investigators, can be ruled out by the absence of metachromasia with or without prior sulfation indicating that the carbohydrate component is of small molecular size with few anionic and hydroxyl groups.

The fluid cement undergoes considerable changes during the hardening process which, in our observations, appears to take place at the site of cement-substratum junction. The mechanism(s) of hardening has not been firmly established. There are some indications, however, that cement hardening agents and/or enzymes are present in the mantle tissue (Shimony and Nigrelli, 1971b).

CONCLUSION AND SUMMARY

On the basis of the selective histochemical tests employed in these studies, it is concluded that the fluid cement (intracellular and intracanicular) in the cyprid and adult stages of the barnacles are collagenous substances. The fluid cement produced by the gland cells of the adult barnacle differs from that formed in the cyprid by the presence of tyrosine, and by a less intense color reaction for carbohydrates. The significance of these differences remains obscure at this time.

The antennular deposits of the cyprid and cement particles in the basal plate produced by the adult barnacle are referred to as solid or hardened cement. The hardened cyprid cement is PAS-positive but non-reactive to acidic and basic dyes; the hardened adult cement is PAS-negative and is stained readily with acidic dyes and, as would be expected, gives a positive reaction for the presence of tyrosyl groups.

APPENDIX

1. Medial Granules: stainable materials found proximal to the nucleus of the secretory cells of the cyprid cement gland.

2. Inner Granules: acidophilic granules found distal to the nucleus of the secretory cells of the cyprid cement gland.

3. Secretory Zones: the acidophilic zones in the adult cement cells where the fluid cement is accumulated for secretion.

4. Collecting Canal: the canal that penetrates the medullary portion of the cyprid cement gland or the canal that is associated with the adult cement cells at the secretory zones.

5. Conducting Canal: the canal that passes within the cyprid antennule and terminates at the attachment pad; in the young barnacle, it is the canal that links the collecting canals of individual cement cells and runs perpendicular to the radial and circular canals.

6. Radial Canals: the two largest canals on the basal plate extending radially along the lateral axis from the center of the basal plate where the adult cement initiates.

7. Circular Canal: the canal that branches out of the radial canal and forms rings on the basal plate. It delivers cement to the exterior through the orifices along the canals.

8. Cyprid Antennular Deposits: the hardened cement which is deposited by the cyprid at the initial point of attachment.

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5

Blood-Group Activity in Baboon Tissues

(Tables 1-4)

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Stomach, salivary-gland, pancreas, and skeletal-muscle tissues from a group-A baboon were extracted with 0.9% saline and 99% ethanol. Only saline extracts from stomach and salivary-gland tissues displayed significant blood-group (group A) activity. Both saline- and ethanol-extracted human group-A_1 erythrocyte stromata, baboon stomach, and baboon salivary-gland tissues displayed similar anti-A-absorption potency in quantitative antibody-absorption-capacity tests. The findings are discussed from the point of view of biochemical evolution, as well as their potential importance in organ-transplantation and cross-circulation procedures.

Introduction

THE A-B-O BLOOD-GROUP STATUS of virtually all of the nonhuman primates has been extensively investigated by Dr. Alexander S. Wiener and his colleagues (Wiener and Moor-Jankowski, 1970). In addition to the elucidation of many fundamental questions regarding the phylogeny of blood groups, such studies have provided the immunohematological basis for the recently developed technique of cross-circulation therapy (Hume et al., 1969).

With this supportive procedure, it has been possible to re-establish homeostatis in human patients (e.g., hepatic coma cases) by utilizing the normal functional capacity of nonhuman primate organs appropriately exchange transfused in advance with compatible human blood. "Consanguinity" at the level of interprimate A-B-O compatibility appears to be the only major tissue-matching prerequisite for this type of supportive therapy. In this regard, group-A and group-B baboons are readily available, and group-O baboons, although of apparently very limited frequency in nature (Wiener and Moor-Jankowski, 1969), could undoubtedly be selectively bred in captivity for cross-circulation procedures requiring group-O compatibility.

One of the unique aspects of A-B-O blood-group expression among the Old World monkeys (e.g., baboons, gelada, and rhesus monkeys), however, is the fact that virtually without exception, A-B-O-active antigens are not detectable as erythrocyte agglutinogens in these primates. Thus A-B-O phenotypes cannot be established directly on the basis of hemagglutination tests (Wiener and Moor-Jankowski, 1970). As a consequence, A-B-O typing of Old World monkeys, which invariably are blood-group-substance "secretors," has been based largely on hemagglutination-inhibition tests performed with boiled samples of saliva (Candela et al., 1940; Wiener et al., 1942). Moreover, except in the serum of gelada monkeys, the specific presence or absence of anti-A or anti-B hemagglutinins has largely followed Landsteiner's rule (Wiener and Moor-Jankowski, 1970), and has thus provided further confirmation for typing results obtained with individual samples of saliva.

In the case of human blood-group-substance "secretors," Beckman (1964, 1970) has observed that, along with their high concentrations of soluble blood-group substances in secretions and other body fluids, secretor types also display elevated serum levels of "intestinal-type" alkaline phosphatase, especially following the ingestion of fatty meals (Langman et al., 1966). It is thus interesting to speculate that other biochemical characteristics, besides merely intestinal-type alkaline phosphatase levels, may prove to be closely associated with the tissue distribution and/or ultrastructural localization of blood-group-active antigens in different species of primates. Indeed, the concept has recently been advanced (Chuba, 1971) that, in one form or another, "blood-group-like" heterosaccharides have been functioning as post-translational "information" molecules vitally involved in the selective transport and binding of substrates throughout organic evolution.

It has already been clearly established that A-B-O-active antigens are ubiquitously distributed throughout both the plant and animal

kingdoms (Springer, 1970; Cushing *et al.*, 1963; Chuba *et al.*, 1971) and are present as alcohol-extractable or water-soluble substances in various tissues besides merely the red blood cells of human beings (Wiener, 1943). Surprisingly few investigations, however, appear to have been undertaken to elucidate the morphogenesis and precise localization of A-B-O-active antigens in various primate tissues and organs (Szulman, 1966). Expanded knowledge in this area would obviously be of basic research interest from the point of view of biochemical evolution, as well as of practical clinical importance from the point of view of organ transplantation (Dausset and Rapaport, 1966, 1968) and cross-circulation therapy (Hume *et al.*, 1969).

The purpose of the present study is to explore the feasibility of investigating the distribution of A-B-O-active antigens in baboon tissues according to: (1) their water-soluble versus their alcohol-soluble properties; and (2) according to procedures adapted from the methodology developed by Basch and Stetson (1962, 1963) to quantitate the tissue distribution of mouse H-2 (histocompatibility) antigens.

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Materials and Methods

Freshly autopsied tissues from a group-A baboon (*Papio anubis*) were provided by the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) of New York University Medical Center. Human erythrocyte stromata (brown preparations) were prepared as previously described (Chuba *et al.*, 1970) or by lysing saline-washed erythrocytes (20% suspensions in 0.9% saline) for several hours at 59°C.

Randomly excised portions of raw baboon tissue (stomach, salivary gland, pancreas, and skeletal muscle) were minced into small fragments in 3 ml of saline per gram of wet tissue for a preliminary 18-hour extraction at 4°C. The once-extracted tissues were then acetone dried at room temperature in large watch glasses, pulverized with a pestle and mortar, and weighed on a fine balance. With the procedure employed, the dry weight of the acetone-dried tissues was consistently some 81% less than the wet weight of corresponding raw tissue, except in the case of pancreatic tissue, where the dry weight was some 90% less than the wet weight.

Portions of pulverized tissue were then re-extracted in saline for 20 hours at 4°C at a concentration of 100 mg of pulverized tissue per ml of saline. Ethanol extracts were obtained by extracting portions of pulverized tissue for 72 hours at 22°C at a concentration of 50 mg of pulverized tissue per ml of 99% ethanol. The saline- and ethanol-extracted substances were then tested according to the procedures described in the footnotes of Tables 1 and 2. Antibody-

Table 1. Hemagglutination-Inhibition Potency of Saline-Extracted Substances from Baboon Tissues.

Saline-extracted tissue (baboon)	Indicator reagents (human) Red cells	Indicator reagents (human) Anti-serum	1:8-titer agglutinating reagent mixed with equal volume of saline extract from											
			Minced raw tissue[1]								Acetone-dried tissue[2]			
			Unboiled and diluted 1:				Boiled and diluted 1:				diluted 1:			
			4	16	64	256	4	16	64	256	4	16	64	256
Stomach	A₁	anti-A	O[3]	O	(O)	++	O	(O)	(O)	++	O	O	(+)	+++
	B	anti-B	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Salivary gland	A₁	anti-A	O	(O)	+++	+++	(+)	+	+++	+++	O	O	(O)	+++
	B	anti-B	+++	+++	+++	+++	ND				++	+++	+++	+++
Pancreas	A₁	anti-A	H[4]	++	+++	+++	+++	+++	+++	+++	O	++	+++	+++
	B	anti-B	C[4]	+++	+++	+++	ND				++	+++	+++	+++
Skeletal muscle	A₁	anti-A	+++	+++	+++	+++	ND				+++	+++	+++	+++
	B	anti-B	+++	+++	+++	+++	ND				+++	+++	+++	+++

ND = not done.

[1] Each gram (wet weight) of minced raw tissue was extracted with 3 ml of 0.9% saline for 18 hrs at 4°C. Inhibition tests were performed with the tissue-free supernate (5000 × G for 5 min.).

[2] Each 100 mg (dry weight) of acetone-dried tissue was extracted with 1 ml of 0.9% saline for 20 hrs at 4°C. Inhibition tests were performed with the tissue-free supernate (1000 × G for 5 min.).

[3] Macroscopic hemagglutination is graded from + to +++; (+) = trace of macroscopic agglutination; (O) = trace of microscopic agglutination; O = no detectable agglutination up to 20X magnification.

[4] H = hemolysis; (H) = partial hemolysis; C = clot formation.

absorption-capacity tests with the extracted tissues were performed quantitatively according to the procedures described in the footnotes of Tables 3 and 4.

All of the serological tests were performed in Kahn-type tubes as previously described (Chuba et al., 1968; Chuba et al., 1970). Hemagglutination reactions were graded after 20 to 30 minute incubation at $22°C$ and a light spin (cf. footnote 3, Table 1).

RESULTS

As shown in Table 1, saline extracts from either raw or acetone-dried stomach and salivary-gland tissues selectively inhibited the hemagglutination of human group-A_1 erythrocytes in reactions which indicated the presence in these tissues of consequential amounts of thermostable group-A substance. The inhibition tests with pancreatic extracts, however, were equivocated by the presence of nonspecific hemolytic activity, which (not shown in any of the tables) also caused the hemolysis of homologous baboon erythrocytes, even when no antiserum was mixed with the pancreatic extracts prior to the introduction of the indicator erythrocytes. Skeletal-muscle extracts, on the other hand, did not display any detectable activity.

As shown in Table 2, none of the ethanol-extracted substances from the baboon tissues displayed consequential blood-group activity in the hemagglutination-inhibition tests. The nonspecific hemolytic activity associated with baboon pancreatic tissue in Table 1, however, was demonstrable in saline suspensions of both the ethanol-extract precipitate and supernate residue derived from the baboon pancreatic tissue in Table 2.

Table 3 shows the quite similar anti-A-absorption potency of human group-A_1 erythrocyte stromata and the baboon stomach and salivary-gland tissues. Interestingly, neither boiling-water-bath treatment (for 15 minutes) nor 72-hour ethanol extraction had a notable effect on the capacity of either the human group-A_1 erythrocyte stromata or the baboon stomach and salivary-gland tissues to absorb human anti-A isoagglutinins.

The nonspecific hemolytic activity associated with baboon pancreatic tissue in Tables 1 and 2 was readily demonstrable in the anti-A serum supernate after absorption with either boiled or unboiled pancreatic tissues (Table 3). Hemolytic activity was not demonstrable in the anti-A serum supernate, however, following absorption with pancreatic tissue from which hemolytic activity had previously been extracted with ethanol in Table 2.

Table 4 shows the disproportionately greater anti-B-absorption potency of human group-B erythrocyte stromata compared with the baboon tissues studied. The weak B-like activity dis-

TABLE 2. HEMAGGLUTINATION-INHIBITION POTENCY OF ETHANOL-EXTRACTED SUBSTANCES[1] FROM BABOON TISSUES.

Ethanol-extracted tissues (baboon)	Indicator reagents (human) Red cells	Indicator reagents (human) Anti-serum	Agglutinating reagent mixed with equal volume of ethanol-derived Precipitate suspension[2] diluted 1: 1	4	16	64	Supernate-residue suspension[3] diluted 1: 1	4	16	64
Stomach	A_1	anti-A	+[4]	+++	+++	+++	+++	+++	+++	+++
	B	anti-B	+++	+++	+++	+++	(+)	+++	+++	+++
Salivary gland	A_1	anti-A	(+)	+++	+++	+++	+++	+++	+++	+++
	B	anti-B	+++	+++	+++	+++	(+)	+++	+++	+++
Pancreas	A_1	anti-A	H[4]	(H)	+++	+++	H	H	+++	+++
	B	anti-B	(+)	+++	+++	+++	H	(O)	+++	+++
Skeletal muscle	A_1	anti-A	+++	+++	+++	+++	+++	+++	+++	+++
	B	anti-B	+++	+++	+++	+++	+++	+++	+++	+++

[1] Substances present in clear (except for pancreas) supernate obtained ($1000 \times G$ for 5 min) from acetone-dried baboon tissue extracted (50 mg dry tissue per ml 99% ethanol) for 72 hrs at $22°C$.

[2] Precipitate was collected ($1000 \times G$ for 1 min) following the additional incubation of the above 72-hr supernate for 48 hrs at $—8°C$. Tests were performed with the acetone-washed precipitate finely suspended in 0.5 ml of saline for each 100 mg of dry tissue originally xtracted with ethanol.

[3] The supernate residue was obtained by evaporating the 48-hr supernate (decanted from the packed $—8°C$ precipitate above) to dryness at $22°C$. Tests were performed with the supernate finely suspended in 0.5 ml of saline for each 100 mg of dry tissue originally extracted with ethanol.

[4] Cf. footnotes 3 and 4, Table 1.

played by the baboon salivary-gland tissue in Table 4, and, to a much lesser extent, by the ethanol-derived supernate residue of baboon stomach and salivary-gland tissues in Table 2, was the only evidence suggesting possible group-B activity in the baboon tissues studied.

As in the case of the absorption of anti-A serum in Table 3, the anti-B serum supernate in Table 4 acquired hemolytic activity during absorption with either boiled or unboiled baboon pancreatic tissue, but not during absorption with pancreatic tissue which had been previously extracted with ethanol. The ethanol-extractable, apparently thermostable hemolytic activity associated with baboon pancreatic tissue in these experiments thus presents a challenging area for further study.

DISCUSSION

The findings with the freshly autopsied baboon tissues are largely consistent with the group-A status previously established for the baboon by LEMSIP investigators on the basis

of: (1) the presence of group-A and absence of group-B activity in saliva samples, and (2) the presence of anti-B and absence of anti-A agglutinins in serum samples studied during the life of the baboon (Dr. W. Socha, personal communication).

The presence of anti-B agglutinins in the serum of the baboon during life indicates that the weak B-like activity of baboon salivary gland tissue in this post-mortem study (Table 4) was not related to B-active receptors possessing the same fine structures as those responsible for group-B activity in human tissues. If the baboon did actually possess weak B-like antigens, notwithstanding the presence of anti-human-B agglutinins in its serum, the situation may be somewhat analogous to the presence of anti-A_1 agglutinins in the serum of certain individuals belonging to the "weak-A" subgroups (Wiener, 1943). In any event, at this point it should be fully appreciated that blood-typing reagents employed to define extrinsic serological attributes or "blood factors" do not, at the same

TABLE 3. COMPARATIVE ANTI-A-ABSORPTION POTENCY OF VARIOUSLY EXTRACTED HUMAN GROUP-A_1 ERYTHROCYTE STROMATA AND BABOON TISSUES.

Absorbing tissue	Mg/ml tissue conc.[1]	Human anti-A serum tested with human group A_1 erythrocytes after absorption[1] with											
		Saline-extracted								Ethanol-extracted tissue			
		Unboiled tissue				Boiled tissue							
		Absorbed serum diluted 1:				Absorbed serum diluted 1:				Absorbed serum diluted 1:			
		1	2	4	8	1	2	4	8	1	2	4	8
None (control)	0	+++[2]	++	+	(O)	+++	++	+	(O)	+++	++	+	(O)
Human grp A_1 red cell stromata	40	O	O	O	O	O	O	O	O	O	O	O	O
	20	+	(O)	O	O	+	(O)	O	O	+	(O)	O	O
	10	+	(+)	(O)	O	+	(+)	(O)	O	+	(+)	(O)	O
	5	+++	+	(O)	O	+++	+	(O)	O	+++	+	(O)	O
Baboon stomach	20	O	O	O	O	O	O	O	O	O	O	O	O
	10	+	(O)	O	O	O	O	O	O	O	O	O	O
	5	+	(O)	(O)	O	(O)	(O)	O	O	O	O	O	O
	2.5	+	(+)	(O)	O	(+)	(O)	O	O	(+)	(O)	O	O
Baboon salivary gland	40	O	O	O	O	O	O	O	O	(O)	O	O	O
	20	(+)	(+)	O	O	+	(O)	O	O	++	(O)	(O)	O
	10	++	+	(O)	(O)	++	+	(O)	(O)	++	++	(O)	(O)
Baboon pancreas	40	H[2]	H	O	O	H	H	O	O	+++	++	O	O
	20	H	H	O	O	H	(+)	(O)	(O)	+++	+++	(+)	(O)
	10	H	++	(O)	O	H	(+)	(+)	(O)	+++	+++	(+)	(O)
Baboon skeletal muscle	80	(+)	(+)	O	O	++	(+)	(O)	O	(O)	O	O	O
	40	++	+	(+)	(O)	++	+	(+)	(O)	+	(+)	(O)	O
	20	+++	+++	(+)	(O)	+++	++	(+)	(O)	+++	++	(+)	(O)

[1] Absorptions were performed by mixing small aliquots of the reagent anti-serum with the number of mg of acetone-dried tissue necessary to provide the mg/ml tissue concentrations specified in column 2 above. Hemagglutination tests were performed with the tissue-free supernate (1000 × G for 5 min.) following 30 min. absorption at 22°C.

[2] Cf. footnotes 3 and 4, Table 1.

time, establish the presence of identical "haptenic" structures in a virtually unlimited number of different substances capable of displaying varying degrees of "blood-group" activity (Landsteiner, 1945; Wiener, 1966).

That baboon and rhesus monkey tissues, for example, may indeed possess a spectrum of B-like receptors slightly different in fine structure from the B-active receptors associated with human blood-group substances is suggested by recent catfish immunization experiments (Chuba *et al.*, 1970). In these experiments, immunization of white catfish (*Ictalurus catus*) with group-B-active baboon or rhesus monkey saliva evoked a complex spectrum of serum heteroagglutinins, not all of which could readily be classified as "anti-B" when tested with human group-B and B-like fur-seal and sea-lion erythrocytes. Brown bullhead catfish (*I. nebulosus*) immunized with human group-B saliva, on the other hand, produced heteroantibodies which, following appropriate absorption fractionation, could be sharply distinguished as anti-B agglutinins (Chuba *et al.*, 1968; Wiener *et al.*, 1968; Chuba *et al.*, 1970).

Not enough catfish of each species were concurrently available for each of the foregoing experiments, however, to establish clearly the extent to which species differences in catfish immune responsiveness, rather than species differences in the B-active antigens used in the experiments, may have significantly influenced the heterogeneity of antibodies produced. Interesting future experiments suggested by the present study would be to inject a series of catfish in parallel with both soluble and particulate antigens from different species of primates. During a preliminary experiment along these lines, Chuba, Kuhns, and Nigrelli (in preparation) and A. S. Wiener (personal communication) found that either boiled saliva, saline-washed erythrocytes, or brown erythrocyte stromata from the same human goup-O secretor, when injected separately into a series of white catfish, evoked virtually the same spectrum of anti-H and anti-Z heteroagglutinins (Wiener *et al.*, 1968; Baldo and Boettcher, 1970; Cushing, 1970) in all of the catfish.

In view of the subgroup polymorphism of animal group-A and group-B antigens (Wiener, 1943), comparative catfish immunization experiments with group-A and group-B antigens derived from different tissues of different species of primates would undoubtedly produce an even more interesting array of catfish heteroagglutinins. Such unique immunological reagents would obviously be of basic research interest, as

TABLE 4. COMPARATIVE ANTI-B-ABSORPTION POTENCY OF VARIOUSLY EXTRACTED HUMAN GROUP-B ERYTHROCYTE STROMATA AND BABOON TISSUES.

Absorbing tissue	Mg/ml tissue conc.[1]	Human anti-B serum tested with human group B erythrocytes after absorption[1] with											
		Saline-extracted								Ethanol extracted tissue			
		Unboiled tissue				Boiled tissue							
		Absorbed serum diluted 1:				Absorbed serum diluted 1:				Absorbed serum diluted 1:			
		1	2	4	8	1	2	4	8	1	2	4	8
None (control)	0	+++[2]	++	+	(+)	+++	++	+	(+)	+++	++	+	(+)
Human grp B red-cell stromata	20	O	O	O	O	O	O	O	O	O	O	O	O
	10	O	O	O	O	O	O	O	O	O	O	O	O
	5	O	O	O	O	O	O	O	O	(O)	O	O	O
	2.5	(O)	(O)	O	O	(O)	(O)	O	O	(+)	O	O	O
Baboon stomach	40	(O)	(O)	O	O	+++	+++	++	(O)	+++	++	+	(O)
	20	+++	+++	+	(+)	+++	+++	++	(+)	+++	+++	+	(+)
	10	+++	++	+	(+)	+++	++	+	(+)	+++	++	+	(+)
	5	+++	+++	+	(+)	+++	+++	++	+	+++	++	+	(+)
Baboon salivary gland	40	O	O	O	O	O	O	O	O	+	(+)	(O)	(O)
	20	++	+	(O)	O	+	(+)	O	O	++	+	(O)	(O)
	10	+++	+	(+)	(O)	+++	++	(+)	(O)	+++	++	(O)	(O)
	5	+++	++	+	(+)	+++	++	(+)	(O)	+++	++	+	(+)
Baboon pancreas	10	H[2]	H	H	H	H	H	(H)	O	+++	+++	++	+

[1] Cf. footnote 1, Table 3.

[2] Cf. footnotes 3 and 4, Table 1.

well as of potential clinical usefulness in tissue-matching procedures.

The negligible effect of boiling-water-bath treatment or ethanol extraction on the capacity of human erythrocyte stromata or baboon tissues to absorb human blood-group antibodies in the present study (Tables 3 and 4) is also noteworthy. It has long been an accepted dualistic concept that the blood-group activity of human erythrocytes is primarily associated with membrane glycolipids and that the blood-group activity of secretions is primarily associated with water-soluble glycoproteins having a carbohydrate content of some 85% (Morgan, 1970; Watkins, 1970). This dualistic concept has been challenged recently, however, with cogent analytical data suggesting that membrane glycoproteins, rather than membrane glycolipids, may actually be primarily responsible for the blood-group activity of human erythrocytes (Whittemore et al., 1969; Poulik and Lauf, 1969; Poulik and Bron, 1970; Zahler, 1968). Our observation that ethanol extraction did not have a notable effect on the residual blood-group activity of the human erythrocyte stromata or baboon tissues studied (Tables 3 and 4) further supports the concept that blood-group-active macromolecules other than alcohol-extractable glycolipids may be primarily responsible for the A-B-O blood-group activity of particulate cellular materials. Moreover, recent investigations which have tended to perpetuate the dualistic concept of cellular-glycolipid versus soluble-glycoprotein blood-group antigens (e.g., Koscielak, 1963) appear to be vulnerable to the criticism that: (1) as already pointed out by Whittemore et al. (1969), only miniscule amounts of blood-group-active glycolipids—quantitatively insufficient to contribute significantly to the blood-group activity of intact erythrocytes—have been extracted from erythrocyte membranes with lipid solvents; and (2) the glycolipid-oriented investigators have invariably failed to assay their "extracted" erythrocyte preparations for residual blood-group activity, such as was done by means of the quantitative antibody-absorption-capacity tests in the present study (Tables 3 and 4).

The possibility thus exists that the cell-membrane-associated glycoproteins include a class of blood-group-active macromolecules possessing physicochemical properties quite different from those of the "water-soluble" blood-group substances. This possibility is supported by the report (Rega et al., 1967) that erythrocyte-membrane glycoproteins have a carbohydrate content of only some 9%. If this proves to be generally true, then membrane-associated glycoproteins, including those with blood-group activity, would presumably be much more readily coagulated and entrapped with other cellular material during various preparatory procedures (e.g., "extraction" with protein-denaturing reagents, etc.) than the "soluble" blood-group substances possessing a carbohydrate content of some 85%. In fact, the demonstration of blood-group activity in baboon stomach and salivary-gland tissues in this investigation could possibly be largely attributable to the in vitro coagulation-entrapment of different classes of blood-group-active glycoproteins, rather than to a preponderance in these tissues of blood-group antigens intrinsically bonded in vivo with the cellular structures themselves. There is also the possibility that substantial fractions of blood-group-active membrane fragments and/or subcellular organelles were not removed with the more particulate tissue debris during routine centrifugation procedures (cf. footnotes, Tables 1 and 2), and thus may have contributed significantly to the "soluble" blood-group activity of the "tissue-free" preparations.

In a classic series of studies, Stetson and his associates quantitated the tissue distribution of mouse H-2 antigens (Basch and Stetson, 1962, 1963), as well as their ultrastructural localization in different membrane fractions and subcellular organelles (Herberman and Stetson, 1965). Similar definitive studies on the tissue distribution and ultrastructural localization of primate A-B-O-active antigens will obviously be necessary before many of the fundamental questions raised by the present study can be fully elucidated.

SUMMARY

1. Stomach, salivary-gland, pancreas, and skeletal-muscle tissues from a freshly autopsied group-A baboon (*Papio anubis*) were extracted with 0.9% saline and 99% ethanol.

2. Only saline extracts from either raw or acetone-dried stomach and salivary-gland tissues displayed significant blood-group (group A) activity in hemagglutination-inhibition tests.

3. Both saline- and ethanol-extracted human group-A_1 erythrocyte stromata, baboon stomach, and baboon salivary-gland tissues displayed quite similar anti-A-absorption potency in quantitative antibody-absorption-capacity tests.

4. In the case of pancreatic tissue, ethanol-extractable, apparently thermostable hemolytic activity interfered with the hemagglutination-inhibition and antibody-absorption-capacity tests.

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6

Captive Breeding of Orangutans

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With more than 500 orangutans in captivity, the world's zoos have a large available breeding stock. The number of births, and the apparent birth rate, have been increasing. However, no living orangutan is the offspring of captive-born parents. The future of the species in captivity depends on successful propagation of the captive born.

THE ORANGUTAN is a symbolic species for wildlife conservationists and zoo directors. Although protected by law wherever it occurs, it was heavily poached for a time. It was one of the few species whose survival might be threatened by collecting for zoos. Tom and Barbara Harrisson, then in Sarawak, called world attention to the illicit traffic and organized the Orangutan Recovery Service which rescued a number of the animals. Mrs. Harrisson undertook the first experiments to determine whether and how captive orangutans might be reintroduced in the wild.

Before 1966, almost every orangutan acquired by zoos was contraband. They were so freely available that negotiating for legal capture and exportation seemed pointless. Then, at the urging of the International Union for Conservation of Nature and Natural Resources, the American Association of Zoological Parks and Aquariums and the International Union of Directors of Zoological Gardens adopted a ban on undocumented orangutans. Other zoo federations took similar action. Import controls were imposed by several nations, while others tried to regulate transit. A few orangutans were seized by authorities. Dealers found fewer customers for smuggled animals, and a number were blocked in the pipeline.

Before the smuggling trade was curtailed, zoo collections were well-stocked. The first official studbook reported 460 as of December 31, 1969 (Bourne, 1971a). The International Zoo Yearbook Census counted 469 a few months later. Two years later, in 1971, the IZY total was 539, but a large part of this increase was caused by reports from additional collections.

[1] Mrs. Horsemen left the Zoo prior to publication.

According to Crandall (1964), the first orangutan brought to Europe arrived in 1640; America saw its first in 1825. Many of the first imports had short lives. Frederick A. Ulmer, Jr. (1966), writes that a Dutchman, Mynheer van Goens, brought 102 Sumatran orangutans to Europe in 1927 and 1928, of which 33 came to the United States in a single shipment. Most soon died. At the Philadelphia Zoo, whose experience with the species extends over almost a century, the average life of 13 animals exhibited between 1879 and 1930 was three-and-one-half years (Ulmer, 1957).

Many of the early deaths were caused by mishandling from capture to delivery. Young orangutans traveling the smuggling route were often in poor condition on arrival. Zoo men gradually learned how to rehabilitate them, and zoo husbandry improved. While Crandall (1964) judged average longevity to be "still rather low" as recently as 1964, there is no reason now to believe captive life-spans are markedly lower than those of free-living orangutans. Philadelphia's "Guas" (Studbook #467) and "Guarina" (Studbook #468) were each about 50 years of age at the end of 1969.

Until longevity improved, little propagation could be expected; but even when adult pairs were kept together, no success was achieved until 1928. In that year, there were captive births at Berlin, Nuremberg, and Philadelphia. While these babies died in infancy, the barrier had been broken. Philadelphia's second orangutan was born in 1930 and successfully reared (Ulmer, 1966).

Until the 1960s, births occurred now and then, with a fair survival rate, but each success was notable. Then came the rapid increase in zoo collections, and an upward trend began.

The IZY reported six successful births in 1964. There were 19 in 1967, 30 in 1971. The IZY census and the studbook report differ with respect to the 1969 total of living individuals that were captive-born: 81 in the census, 101 in the studbook. In 1971, according to IZY, there were 152 captive-born orangutans. Only 13 percent of those reported in the 1964 census were captive born. There were 28 percent in 1971.

The studbook provided the first data with which to measure breeding potential and results. The first edition gave information on all births occurring in 1967, 1968, and 1969, and on all orangutans reported in collections as of December 31, 1969 (Bourne, 1969).

Captive female orangutans are most likely to bear offspring between the ages of seven and eighteen, although Philadelphia's "Guarina" delivered nine babies between 1929 and 1955, a reproductive period of 26 years (Ulmer, 1966).

During the three years of the studbook reports, 122 females were in the 7 to 18 age group, and 45 of them produced at least one live infant, 37 percent of those eligible. The 45 had a total of 65 successful births, including one set of twins. There is no reliable way to compare propagation in the wild. Barbara Harrisson (1971, private correspondence) notes that present-day orangutan habitats differ greatly in quality, with consequent effects on life spans, propagation, and infant mortality. She believes wild females seldom bear young before nine years of age. Infant mortality tends to be rather high in the wild, ranging around 40 percent. A stable population in a protected habitat would indicate that the average female produces four to five babies, usually between the ages of nine and 30-plus (Harrisson, 1971, private correspondence).

If "Guarina" were typical of captive females, the comparison would be favorable. Of her nine offspring, four were living at the end of 1969. But only two other captive females had this many surviving offspring, and only five had three surviving offspring (Bourne, 1971a). No data is available on the number of deceased offspring.

A rising percentage of captive-born orangutans would seem reassuring. It may not be. If, for example, accessions of wild-caught stock ceased and second-generation births fell short of the replacement rate, the percentage of captive-born individuals would approach 100 percent as the total population approached zero. The wild-caught individuals, being older, would usually die before their progeny.

The number of births has risen from year to year. It is disquieting, however, that no living orangutan is the offspring of two zoo-born parents[1]. Before the studbook appeared, we heard rumors of second-generation and even third-generation births. In part, this may have been misunderstanding. Two correspondents used "second generation" to mean the first zoo-born generation.

Since Philadelphia was the site most named in the rumors, we wrote to Fred Ulmer. His reply: In every Philadelphia orangutan birth, at least one parent was wild-caught. He added as a footnote: "We did have some breeding between 'Pinky' and 'Ivy', who are brother and sister, but the matings always resulted in abortions" (1971, private correspondence).

Has there yet been time for second-generation births to occur? The number of captive-born individuals has increased slowly since 1930. A few captive-born females have given birth, and a few captive-born males have sired young. The studbook does not disclose how many captive-born pairs of breeding age have been kept together.

Of the 122 females of breeding age during the 1967-1969 period, 109 were wild-caught, only 13 zoo-born. Of the 64 females then in the 9 to 11 age classes (the most productive years, according to studbook records), only five were zoo-born. Obviously the potential for second-generation propagation is still low. Further, many zoo men prefer to pair captive-born individuals with wild-caught mates, or they do so simply because captive-born mates are not available. At Philadelphia, for example, "Ivy," born to "Guas" and "Guarina" in 1937, was mated with her father and produced a female baby in 1950.

Still, there is reason for concern, and this will not be eased until a satisfying number of second and third generation births have occurred. Are there problems and challenges to be overcome by management skill?

There is evidence that behaviors such as mating and maternal care are, to some degree, learned by the great apes in their natural settings. While this is more pronounced among gorillas than orangutans, observations in a number of zoos indicate the latter is influenced. A number of zoo-raised male orangutans have, on reaching sexual maturity, responded to the sexual urge in ways more bizarre than appropriate. A number of zoo-raised females have, on giving birth, rejected their infants. Dr. Lang (1971) comments: "Orangs tend to be less affected than gorillas when deprived of social experience with their own kind in infancy, but nevertheless the

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[1] A second-generation birth occurred at Rotterdam some years ago, according to Marvin Jones. It died prior to 1967.

number of failures in maternal behavior must give rise to some concern."

When the Wild Animal Propagation Trust was organized in the United States to promote captive breeding of endangered species, the Orangutan Committee was the first named. One of the needs was to bring together unpaired males and females. Thanks to the cooperation of a number of zoos and dealers, this has been reasonably successful. At the time of the 1971 IZY census, only eight U.S.A. collections had one sex only, and seven of these had males, for which no females had been found.

It has also been the experience of some zoos that males and females raised together failed to mate on becoming sexually mature. The National Zoo had such a pair. In cooperation with WAPT, our male was sent to the Cheyenne Mountain Zoo in Colorado, where he sired young; we obtained another male which promptly mated successfully with our female. WAPT has helped to arrange other transfers and deposits.

Such arrangements are not easily made, however. Orangutans are much prized as exhibits, and directors of city-owned zoos are often not free to dispose of valuable animals. Even when a zoo director can subordinate local interests, finding a proper match is often difficult. The IZY census is not current when published, and it does not report the ages of individuals. The studbook provides more data but it has not been issued since 1969. No service provides information on the births, deaths, accessions, and removals that occur from month to month.

Dr. Bourne of the Yerkes Regional Primate Research Center writes (1971b): "I believe the key to sustained breeding is a large group of animals, so that switching of males and females can occur to be sure you can get compatible breeding pairs."

If this is the key to sustained breeding, the present outlook is not favorable. At the time of the 1971 IZY census, the world's largest orangutan collection was at Yerkes, with 28 individuals, plus seven on loan to the nearby Atlanta Zoo. Second largest outside Indonesia, 14 individuals, was at the Cheyenne Mountain Zoo. But the average number in 128 zoos reporting the species was only four. In such small groups, there are sure to be less than the optimum number of compatible adult pairs.

A high proportion of young orangutans are raised in isolation after they are taken from their mothers. Many are taken shortly after birth. Some zoo men are concerned lest the hand-raising of zoo-born orangutans further complicate the breeding problem. Many infants are handled like human babies, associating only with humans in the early months of life. When they are returned to the zoo, it is usually to a lonesome cage, since there are long odds against the zoo having a compatible cage-mate. The managers of some collections advocate hand-raising and, indeed, remove all infants as a matter of course, regardless of whether the mother seems capable of providing care.

The Yerkes laboratory has the advantage Dr. Bourne cites in its number of possible mating combinations. Like most other collections, however, it provides caging for individuals or pairs. In these circumstances, there is little opportunity for the kind of behavioral learning mentioned by Dr. Lang. Only in recent years have ideas or plans been put forward to experiment with groupings of orangutans in large enclosures.

One of the authors collaborated with Barbara Harrisson in designing an experimental enclosure. This called for several open-frame towers, 30 to 40 feet tall, with several platforms, each of which would shade the zone below. Orangutans would be fed in the towers. The hope was that the towers would serve as vertical territories. This design was followed in new construction at the Surabaja and Djakarta zoos (Indonesia). The young orangutans are climbing and brachiating as expected, but the real test will not come until they are sexually mature.

In the United States, some decisions about caging must soon be made, for there is an acute space shortage. Many juvenile orangutans are kept in quarters inadequate for adults, and new construction is lagging behind the needs of this maturing population.

The National Zoo was compelled to dispose of a young pair, through WAPT, for lack of space. In this case, WAPT was able to place them advantageously, but such placements are becoming more difficult.

In summary, it is far from certain that the captive orangutan population will become self-sustaining. The number of births and the birth rate[2] will probably increase for several more years, simply because more wild-caught females will be entering the period of maximum fecundity. In these years, births may exceed deaths, so that the captive population increases. There may be a surplus of orangutans in zoos, as available quarters become crowded.

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[2] Birth rates are unreliable indicators in a population as small and changing as this one. Based on IZY data, captive orangutan births per 1000 population have been:

1964	22	1968	48
1965	26	1969	62
1966	54	1970	64
1967	43	Cumulative average: 48	

This increase can be misleading, however. The future of the captive population depends on births in the second and succeeding captive generations. It is too soon to predict that these will not occur in sufficient numbers, but also too soon to say that they will.

Predictions are likely to be unreliable unless better data is developed for analysts to ponder. It is disturbing that the studbook data is not sufficient. One cannot evaluate second-generation birth results without knowing how many first-generation pairs have been given the opportunity of mating. It would also be valuable to known which captive-born individuals were hand-raised.

Dr. Bourne finds reason for cautious optimism in success with chimpanzees (1971b, private correspondence):

> One of our chimpanzees has five great-grand-children living at the Center, and another animal has two great-great grandchildren. The last two are the product of two successive captive born matings for sure . . . This is all very good, and although we cannot necessarily extrapolate to orangs, it is encouraging.

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Captive Propagation: A Progress Report

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Of 291 mammals species and subspecies listed by IUCN as rare or endangered, 162 have been reported in zoo collections since 1962, and 73 have reproduced at least once. Only a few of these, however, have captive populations which seem reasonably secure in terms of numbers and reproduction rates.

THE FIRST ZOO TO PROPAGATE a species in captivity earns a mark of distinction. In recent years, there have been fewer such events than in the past. Many species once thought impossible to breed in captivity have been bred. Others that reproduced rarely now do so more often.

On balance, zoos are still consumers rather than producers of wildlife. A few zoo directors have protested this statement, but available vital statistics confirm it. A typical report from a leading zoo shows:

	Births & Hatchings	Deaths	Net
MAMMALS	156	178	− 22
BIRDS	252	433	−181
REPTILES, AMPHIBIANS, ETC.	0	318	−318

Further, births and hatchings are not evenly distributed over the species in a collection. Among the birds, for example, a few species usually account for most hatchings.

A few zoos are net producers. By and large, these have specialized in their collections. Game parks, game farms, and establishments devoted to breeding waterfowl or upland game birds usually produce annual surpluses.

That zoos might become survival centers for endangered species is not a new idea. Proposing that a national zoo be established, in 1889, Smithsonian Secretary Samuel P. Langley declared it would be "a home and a city of refuge for the vanishing races of the continent." As more and more species approach extinction, interest in survival centers has increased. Citing the Przewalski horse and wisent as examples, some zoo directors assert that captive breeding will be the last hope for many species.

It seems timely to consider what has been accomplished thus far. Because the preceding table is typical, we have limited this review to mammals. The IUCN Red Data Book lists 291 mammal species and subspecies as rare or endangered. In 1962, the International Zoo Yearbook undertook the first of its annual censuses of rare species in zoos. Since that time, 162 of the 291 species and subspecies have been represented in collections. IZY reports of births indicate that 73 of these produced offspring at least once in the ten-year period.

To simplify analysis, we chose two base years, 1962 and 1965, and from the 73 species and subspecies selected those with captive populations of ten or more in either year. This is a crude method of choice; a herd of eight could be a good breeding base, while two dozen widely scattered would not be. However, on reviewing the species and subspecies thus eliminated, we saw no serious omissions for purposes of this study.

There were 41 mammal species and subspecies with base-year populations of ten or more. The 1971 IZY Census showed population increases for 36 of them. This is not, in itself, evidence of breeding success, since the IZY Census does not report acquisitions from the wild. Further, the number of zoos reporting to IZY increased.

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[1] Mrs. Horsemen left the National Zoological Park prior to publication.

IZY does report the numbers of captive-born individuals within each year's totals. When this data is assembled, there are strong indications of whether a captive population is self-sustaining.

(In the following tables, a blank for 1962 may mean zero response. However, some species and subspecies have since been added to the Census list.)

ZOO POPULATIONS OF RARE AND ENDANGERED SPECIES: 1962-1971

	1962	1965	1971	Captive-born No.	Percent
MARSUPIALIA					
Yellow-footed Rock Wallaby *(Petrogale xanthopus)*	4	52	46	42	91
Long-nosed Rat Kangaroo *(Potorous tridactylus)*	5	13	23	8	35
White-throated Wallaby *(Macropus parma)*	—	19*	180	70	39
*Not reported by IZY in 1965. Data for 1966.					
PRIMATES					
Black Lemur *(Lemur macaco)*	32	25	73	28*	38
Red-fronted Lemur *(Lemur fulvus rufus)*	3	10	43	15*	35
*Number of captive-born not reported by Tananarive.					
Mongoose Lemur *(Lemur mongoz mongoz)*	22	59	167	64	38
Red Uakari *(Cacajao rubicundus)*	8	32	38	4	11
Goeldi Monkey *(Callimico goeldii)*	—	10**	16	6	38
**Not reported by IZY in 1965. Data for 1967.					
Golden Lion Marmoset *(Leontopithecus rosalia)*	—	72***	76	39	51
***Not reported by IZY in 1965. Data for 1966.					
Orangutan *(Pongo pygmaeus)*	205	349	539	152	28
Bonobo Chimpanzee *(Pan paniscus)*	9	22	21	4	19
CARNIVORA					
Maned Wolf *(Chrysocyon brachyurus)*	7	11	65+	22	34
Spectacled Bear *(Tremarctos ornatus)*	13	43	85+	16	19
Brazilian Otter *(Pteronura brasiliensis)*	10 min	10 min	15	3	20
Brown Hyena *(Hyaena brunnea)*	5	32	49	17	35
Asiatic Lion *(Panthera leo persica)*	3	37	66+	22+	33
Siberian Tiger *(Panthera tigris altaica)* (Includes Korean form)	—	120	296+	153+	52
Sumatran Tiger *(P. t. Sumatrae)*	—	23	78+	59+	76
North China Leopard *(Panthera pardus japonensis)*	—	29	51+	44+	86
Snow Leopard *(Panthera uncia)*	22 min	54	98	31	32

	1962	1965	1971	Captive-born No.	Percent
PERISSODACTYLA					
Przewalski Horse *(Equus przewalskii)*	85 min	121 min	182	181	99
Onager *(Equus heminonus onager)**	62	113	139+	74	53
*Including animals reported as E. h. heminonus. This combination was initiated by IZY in 1966.					
Indian Wild Ass *(E. h. khur)*	3	11	11	1	9
Nubian Wild Ass *(Equus asinus africanus)*	7	16	17	17	100
Hartmann Mountain Zebra *(Equus zebra hartmannae)*	54	72	91+	37+	41
Baird Tapir *(Tapirus bairdii)*	6	11	12+	2	17
Great Indian Rhinoceros *(Rhinoceros unicornis)*	26	39	45+	16	36
Black Rhinoceros *(Diceros bicornis)*	119	124	128+	28	22
ARTIODACTYLA					
Pygmy Hippopotamus *(Choeropsis liberiensis)*	49	85	128+	58+	45
Vicuna *(Vicugna vicugna)*	—	72	69	57	83
Burma Brow-antlered Deer *(Cervus eldi thamin)*	13	11	37	5	14
Thailand Brow-antlered Deer *(C. e. siamensis)*	—	12*	10	9	90
*Paris Zoo herd identified as C. e. eldi in IZY 1965.					
Tule Elk *(C. canadensis nannodes)*	—	14	32	17	53
Formosan Sika *(C. nippon taiouanus)*	—	306	374+	336+	90
Pere David Deer *(Elaphurus davidianus)*	130	436	550	550	100
Anoa *(Anoa depressicornis)*	—	23	24	7	29
Wisent *(Bison bonasus)*	132	234 min	303+	232+	77
Arabian Oryx *(Oryx leucoryx)*	5	27	75+	49+	65
Scimitar-horned Oryx *(Oryx tao)*	18	23	141	101	72
Addax *(Addax nasomaculatus)*	20	63	142	116	82
Arabian Gazelle *(Gazella gazella arabica)*	—	10 min	44+	19+	43
Ovis orientalis omitted because of apparent changes in subspecies identification.					

These 41 cases include a wide range of situations. The Przewalski horse story is familiar. At the other extreme, the Brazilian otter is obviously insecure. In between are a number of species which show promising trends but, as yet, provide more reason for hope than confidence.

Our purpose was to identify those situations where zoo propagation has been sufficient to give reasonable assurance that a species can be permanently maintained without further acquisitions from the wild. As a beginning, we chose two arbitrary factors: a 1971 captive population

of 100 or more, and at least half of these captive-born. While these factors alone could not guarantee long-term security, it is unlikely that anything less would.

Using these two factors as a screen, only eight species or subspecies qualified: the Siberian tiger, Przewalski horse, onager, Formosan sika,

Pere David deer, wisent, scimitar-horned oryx, and addax. The mongoose lemur *(Lemur mongoz mongoz)* is a possible candidate for this list; of the two principal collections, one did not report, while the second did not report numbers of captive-born.

1. SIBERIAN TIGER	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	36	41	49	50	51	66	71	77
Total population	104	116	149	162	191	224	248	296
Captive bred	73	66	87	109	140	161	192	253
Percent captive bred	70	57	58	67	73	72	77	85
Births (surviving)	21	28	28	43	58	59	75	—*
Individuals per collection	3	3	3	3	4	3	3	4

*IZY reports births for the year preceding the Census.

The number of individuals per collection remained almost static during the years the population increased by 185 percent. The number of births increased slightly more rapidly than the total population.

In the years shown, 312 successful births were

reported. The total population increased by 192, the population of captive-born individuals by 180. The number of wild-caught individuals increased from 31 to a peak of 63 in 1969, and has since declined to 43. The apparent birth rate has increased.

2. PRZEWALSKI HORSE	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	24	29	33	35	40	41	43	42
Total population	118	121	149	147	157	160	161	182
Captive bred	116	120	148	146	156	159	160	181
Percent captive bred	98	99	99	99	99	99	99	99
Births (surviving)	18	12	18	19	19	14	27	—
Individuals per collection	5	4	5	4	4	4	4	4

This species is often mentioned as a prime example of survival in zoos. The total population has shown a slow but steady increase. The

number of zoos having the species has also increased. The average number of individuals per collection remained constant.

3. ONAGER*	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	26	33	38	39	32	37	43	44
Total population	89	113	135	150	118	132	145	139+
Captive bred	34	34	61	76	65	56	77	74
Percent captive bred	38	30	45	51	55	42	53	53
Births (surviving)	6	18 min	15	14	13	14	14+	—
Individuals per collection	3	3	4	4	4	4	3	3

*Includes animals once reported as *E. h. hemionus.*

An apparent population decline occurred in 1968. While there were reporting inconsistencies, losses were also indicated, and the population total has yet to regain its 1967 peak, nor has the total of captive-born individuals.

The average number per collection has re-

mained almost static, as has the apparent birth rate. Of the 44 collections reporting in 1971, 11 had only one sex.

The onager position is not yet secure, though there is no immediate reason for alarm.

4. FORMOSAN SIKA	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	21	33	32	29	26	26	24	30
Total population	135	306	260	327	420	414	539	374
Captive bred	113	189	209	234	233	248	361	336
Percent captive bred	84	62	80	72	55	60	67	90
Births (surviving)	49	46	65	70	38	52 min	68	—
Individuals per collection	6	9	8	11	16	16	22	12

There appear to be problems of subspecies identification here. Mountain Home (Texas), a private game ranch, reported 105 *Cervus nippon taiouanus* in 1970, none in 1971. However, it reported 60 *C. n. mantchuricus*, all captive-born, in 1970 and an estimated 200 in 1971. The total population shown for 1971 was further affected by lack of a report from Taipeh, which had re-

ported an estimated 150 in 1970.

Total population in all other collections increased by 90 from 1970 to 1971. The average number per zoo declined from 15 to 12; the number of collections increased from 22 to 30.

This subspecies appears to be in a strong position for long-term survival in captivity.

5. PERE DAVID DEER	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	43	44	45	49	51	54	60	63
Total population	410	432	436	452	485	497	525	550
Captive bred	410	432	436	452	485	497	525	550
Percent captive bred	100	100	100	100	100	100	100	100
Births (surviving)	97	87	104	120	102	27	99	—
Individuals per collection	10	10	10	9	10	9	9	9

The apparent decline in births for 1969 was caused by a lack of report from Woburn.

IZY now reports only totals for this species, not individual zoo data, which is available from the studbook. In 1968, last year for the indi-

vidual reports, 60 percent of the population was at Woburn.

This species appears to be in a reasonably secure position.

6. WISENT	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	45	58	64	61	70	82	74	76
Total population	177	234	248	258	249	281	283	303
Captive bred	146	145	154	182	192	193	212	232
Percent captive bred	82	62	62	71	77	69	75	77
Births (surviving)	24 min	31	34	30	47	51	44	—
Individuals per collection	4	4	4	4	4	3	4	4

The total population has increased, the average per collection remaining static. Though the

population increase has been slow, the species seems secure.

7. Scimitar-horned Oryx	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	7	11	9	10	14	18	23	25
Total population	11	23	22	27	53	92	ca.125	141
Captive bred	8	16	16	15	14	44	73	101
Percent captive bred	73	70	73	56	26	48	58	72
Births (surviving)	4	4	8	5	26	23	29	—
Individuals per collection	2	2	2	3	4	5	6	6

The captive population of the scimitar-horned oryx has increased almost explosively since 1967, leaping from 27 to 141 individuals. From 1967 to 1968, the wild-caught population increased from 12 to 39, reaching a peak of 52 in 1970. Since 1968, the number of captive-bred individuals has risen from 14 to 101, and the percentage of captive-bred individuals has been rising rapidly. The average number of individuals per collection has also increased. If the trends continue, this species will be in a strong position for the future.

8. Addax	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	12	17	18	19	17	21	24	27
Total population	59	63	55	72	75	93	126	142
Captive bred	30	42	32	42	31	58	81	116
Percent captive bred	51	67	58	58	41	62	64	82
Births (surviving)	8	16	15	18	·24	29	29	....
Individuals per collection	5	4	3	4	4	4	5	5

The reported wild-caught population has fluctuated from year to year, reaching a peak of 45 in 1970, declining to 26 in 1971. The captive-bred population has increased rapidly since 1968. While this species has not yet attained the total numbers of the wisent or Pere David deer, the position is becoming stronger.

*   *   *

In seven of these eight cases, captive breeding seems to have established reasonable security for the species, or nearly so. It is interesting that seven of the eight are hoofed animals, which require more zoo space than most smaller mammals.

When the zoo-by-zoo data is analyzed, it appears that the collections with the largest numbers of a species tend to produce disproportionately large shares of the births. One reason for this is that the general averages are depressed by the number of collections having only one sex. In a number of cases, an increase in the number of collections is accompanied by an apparent decline in the average birth rate. This may be because a collection just acquiring the species may not have both sexes or it may have acquired a pair not yet of breeding age.

Among the 33 other species in the initial table, a number show promising population increases. Five have total populations of more than 100. For nine others the percentage of captive-bred exceeds 50. We have chosen nine additional cases from the 33, not by formula but because of their special interest:

1. Golden Marmoset	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	ND	ND	23	27	28	24	23	20
Total population	ND	ND	72	99	102	96	84	76
Captive bred	ND	ND	6	8	19	22	34	39
Percent captive bred	ND	ND	8	8	19	23	40	51
Births (surviving)	5 min	7	5	10	10	18	11	—
Individuals per collection	ND	ND	3	4	4	4	4	4

ND＝No data available

The population has decreased since 1968. While the percentage of captive-bred individuals has risen sharply, this is not in itself a hopeful sign. Since imports of new stock have been cut off, this percentage could rise to 100 percent while the number in captivity approached zero.

2. ORANGUTAN	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	96	107	120	141	120	119	117	128
Total population	278	349	389	438	434	455	469	539
Captive bred	37	44	46	55	68	81	112	152
Percent captive bred	13	13	12	13	16	18	24	28
Births (surviving)	6	9	21	19	21	28	30	—
Individuals per collection	3	3	3	3	4	4	4	5

The apparent population increase of 70 in 1971 was largely caused by reporting incongruities.

The percentage of captive-born individuals has been rising slowly, as has the number of births. The apparent birth rate has remained relatively stable since 1966.

Many wild-caught orangutans were acquired within a few years preceding 1967. The wild-caught population outside Indonesia reached a peak in 1967 and is now slowly declining. Thus far, captive births have more than offset this decline, but it will be several years more before the likelihood of survival in captivity can be assessed.

3. SUMATRAN TIGER	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	20	11	27	34	30	30	28	29
Total population	44	23	50	86	66	65	62	78
Captive bred	24	5	24	42	42	42	48	59
Percent captive bred	55	22	48	49	64	65	77	76
Births (surviving)	1	6	7	18	12	9	12	—
Individuals per collection	2	2	2	3	2	2	2	3

While there have been reporting inconsistencies, the population decline following the 1967 peak seems to be real. The wild-caught total has declined from a peak of 44 to 19. The number of births has not significantly increased. The apparent birth rate over seven years has been substantially below that of the Siberian tiger.

4. SNOW LEOPARD	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	27	28	28	33	39	42	39	44
Total population	49	54	54	64	90	96	93	98
Captive bred	8	4	3	15	15	20	29	31
Percent captive bred	16	7	6	23	17	21	31	32
Births (surviving)	3	1	6	15	7	10	7	—
Individuals per collection	2	2	2	2	2	2	2	2

The population of this species has increased chiefly through acquisitions from the wild. Only a modest increase has occurred since 1968. The number of births does not show an upward trend. The average number per collection has remained static, at two. Of the 44 collections, 11 had only one sex in 1971.

5. HARTMANN MOUNTAIN ZEBRA	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	21	20	22	28	28	33	34	29
Total population	80	72	78	86	81	94	84	91+
Captive bred	21	24	32	35	36	38	42	37+
Percent captive bred	26	33	41	41	44	40	50	41
Births (surviving)	9	7	14	10	9 min	9 min	10	—
Individuals per collection	4	4	4	3	3	3	2	3

Total population has fluctuated only slightly during this period. The captive-bred numbers have changed only slightly since 1967. Of the 29 collections, 10 have only one sex. While 68 successful births were reported, the captive-born population increased by only 16.

6. BLACK RHINOCEROS	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	63	66	72	68	67	72	71	65+
Total population	113	124	132	126	126	136	130	128+
Captive bred	18	16	23	21	21	24	27	28
Percent captive bred	16	13	17	17	17	18	21	22
Births (surviving)	2	6	1	3	6	7	9	—
Individuals per collection	2	2	2	2	2	2	2	2

Total population fluctuated only slightly during the period. There was a modest increase in the number and percentage of zoo-born individuals. While 34 successful births were reported, the zoo-born total increased by only 10. The average number per collection remained static. Of the 65 collections reporting in 1971, 25 had only one sex.

7. PYGMY HIPPOPOTAMUS	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	31	33	38	44	43	47	46	48
Total population	80	85	99	103+	108	124	126	128+
Captive bred	35	38	42	45	44	55	50	58+
Percent captive bred	44	45	42	44	41	44	40	45
Births (surviving)	6	12	7	3	11	5	8	—
Individuals per collection	3	3	3	2	3	3	3	3

This species came close to the arbitrary selection factors: 100 or more individuals, 50 percent or more captive-born. In the period shown, the captive population increased by 48, the captive-born total by 23. The number of births reported during the period was 52.

The percentage of captive-born individuals remained remarkably static. Births averaged 7.4 per year, the actual number fluctuating from year to year. The apparent birth rate tended to decline. The average number per zoo remained static.

8. VICUNA	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	28	32	ND	ND	23	20	22	22
Total population	69	72	ND	ND	64	70	70	69
Captive bred	34	38	ND	ND	43	53	44	57
Percent captive bred	49	53	—	—	67	76	63	83
Births (surviving)	7	4	7	4	10	3 min	5	—
Individuals per collection	2	2	—	—	3	4	3	3

The percentage of captive-bred individuals has risen, but total population has not increased. The number of births has fluctuated from year to year. Nine of the collections have only one sex.

9. Arabian Oryx	1964	1965	1966	1967	1968	1969	1970	1971
No. zoos reporting	4	4	5	5	6	5	4	4
Total population	27	27	39	49	44	48	58	75
Captive bred	2	7	10	9	9	18	18	49
Percent captive bred	7	26	26	18	20	38	31	65
Births (surviving)	2	3 min	3 min	1	5	4	6	—
Individuals per collection	7	7	8	10	7	10	15	19

The increase from 1970 to 1971 in the number of captive-bred individuals is distorted by the report from Qatar, which failed to report this item in 1970, but reported 21 captive-bred animals in 1971. The significant increase is in the captive-bred totals for Phoenix and Los Angeles: from 18 in 1970 to 28 in 1971. For these two collections alone, the percentage of captive-bred individuals was 62 in 1970, 74 in 1971.

The total captive population has increased rapidly, with a significant increase in the average number per collection. Births show an upward trend.

* * *

On the record to date, zoos have not become a significant resource in the preservation of rare or endangered mammals. Seven species or subspecies endangered or extinct in the wild appear to have reasonably secure captive populations with potential for reintroduction. In a few other cases, favorable trends give promise of security in the near future. While these are important contributions, their number is small by comparison with IUCN's long and growing list.

The data also indicate that zoos can become a more significant resource. The chief deficiency is managerial, not scientific. Zoos have learned, over the years, how to keep most species alive and healthy in captivity, and how to breed them. Many of these species would undoubtedly multiply to satisfactory numbers if adequate breeding groups were brought together under proper conditions. While some species now present special problems, such as inadequate second-generation reproduction, most should be responsive to concerted efforts.

The troublesome problem is that many species which reproduce adequately under good management do not have self-sustaining captive populations.

That many zoos report only single males or single females is only part of the problem. A zoo with one of each does not necessarily have a breeding pair. The problem centers in the zoos that do the best job of propagation but, for lack of space, are compelled to dispose of offspring. Too often these offspring are sent to zoos with lesser resources and qualifications, zoos that may wish them only for exhibition.

A breeding pair may produce offspring for several years. Then, if the male or female is lost, no replacement may be readily at hand. Further, many zoo directors report difficulty in finding takers for their surplus. They might prefer to send their animals to excellent zoos that emphasize breeding, but such discrimination may not be possible. One zoo deliberately prevented matings of an endangered species because its pens were overcrowded by the previous year's surplus.

The capacity of zoos is limited, and most still emphasize diversity in collections. A random selection of ten leading zoos shows an average number of individuals per mammal species of 3.9, the range being from 3.1 to 4.6. Since this average includes over-age individuals, non-breeders, and juveniles, there are, inevitably, many situations without breeding potential.

Increased propagation of endangered species is feasible, but it may be stifled or become futile unless progeny can be accommodated in their natal zoos or in others willing and able to further propagation. Room for growing numbers must be found, either by displacing more common species or by establishing rural survival centers.

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8

Status of Rare and Endangered Birds in Captivity with a General Reference to Mammals

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Of the 340 bird forms reported by the IUCN as rare and endangered, 62 were reported in captivity from 1964 to 1970. Among the 62 captive forms, 30 bred once or more in captivity but only 24 bred with frequency. Significant captive breeding success occurred primarily in the Anseriformes, Galliformes, and Psittaciformes. Only nine forms appear secure with regard to captive numbers and reproductive rate.

Introduction

VIRTUALLY EVERY ZOO has repeatedly stated as a major objective the captive propagation of endangered species as a mechanism for saving such animals from extinction. This goal has served as a justification for the possession of endangered animals. The International Union for the Conservation of Nature and Natural Resources (IUCN) has endorsed this principle as one viable alternative to extinction. This paper represents an effort to evaluate the captive status of rare and endangered birds from 1964 through 1970 currently listed in the IUCN Red Data Book. A general analysis is given for mammals during the same period.

Methods

Data for each form listed in the current bird and mammal Red Data Book lists were compiled from the International Zoo Yearbook's (IZY) lists of rare and endangered animals and animals bred in captivity, Volumes 5 through 11. Areas examined for each species were: 1) number of exhibiting zoos; 2) number of zoos reporting births or hatchings; 3) total species number; 4) total number captive born surviving; 5) number born for each year; and 6) number of zoos possessing a breeding potential. Although each year was analyzed, the tables of this paper were prepared using two-year intervals. Rather than indicate sex, a number representing reproduction potential is used. It is derived for each species by counting the number of zoos indicating the possession of both sexes and/or breeding success. When this number is compared to the number of births and number of young born, a statement of trend can be made. Birth data is

always given for the year preceding the yearbook volume and has been adjusted accordingly.

It must be recognized that figures presented here are in many cases incomplete or inaccurate due to several reasons, including 1) irregular reporting by institutions; 2) taxonomic misidentification; 3) failure to provide numerical assessments for individual species; 4) failure of some countries to report; 5) lack of information from private breeders; 6) lack of information on ages of stock; and 7) lack of consistent survival criteria. Because of these factors, the data presented here are simply indicators of trends, but it is felt that they do reflect with some accuracy the captive status of rare and endangered birds and mammals in major zoo facilities from 1964 through 1970.

Aves

General Comments. For the 27 orders of birds, seven (Struthioniformes, Rheiformes, Casuariiformes, Apterygiformes, Gaviiformes, Coliiformes, and Trogoniformes) contain no forms considered rare and endangered for the purposes of this paper (Table 1). The remaining 20 orders contain 340 rare and endangered forms. Nine orders were represented in captivity by 62 forms (18.2 percent of all endangered forms). Six orders (Ciconiiformes, Anseriformes, Galliformes, Gruiformes, Psittaciformes, and Passeriformes) contain 30 breeding forms which is 48.9 percent of all forms exhibited, but only 8.8 percent of the total number of rare and endangered species. For the 30 breeding forms represented in captivity, only 24 have bred with any consistency and quantity. Rare and endangered forms were represented in captivity in three additional orders (Sphenisciformes, Fal-

coniformes, and Columbiformes) but no breeding was reported.

Those orders containing rare and endangered forms which have been reported in captivity during 1964 through 1970 are reviewed below.

Sphenisciformes. The Galapagos penguin is the single Red Book representative and was infrequently reported in captivity, only once with breeding potential.

Ciconiiformes. Five forms are rare and endangered (4 sp., 1 ssp.). The Japanese white stork data is incomplete, lacking in data from Chinese zoos. A captive breeding project in

Japan has so far not proven successful. The red-cheeked ibis or waldrapp (Table 2) is reproducing primarily from two large groups at Basle and Innsbruck.

Anseriformes. Thirteen forms (9 sp., 4 ssp.) are rare and endangered and nine were reported in captivity. Eight bred frequently, while the white-winged wood duck reported only at Slimbridge in 1964, 1969, and 1970, has been less than successful. Table 3 indicates status of the remaining eight forms. The Hawaiian goose is a classic captive-breeding success story (Fisher et al., 1969), with stock, breeding potentials,

TABLE 1. *Ordinal Summary of Captive Status for Rare and Endangered Birds — 1964-1970

Order	IUCN Forms	Forms in Captivity	Rarely	Captive Breeding Frequent	Total
Sphenisciformes	1	1	0	0	0
Struthioniformes	0	0	0	0	0
Rheiformes	0	0	0	0	0
Casuariiformes	0	0	0	0	0
Apterygiformes	0	0	0	0	0
Tinamiformes	2	0	0	0	0
Gaviiformes	0	0	0	0	0
Podicipediformes	5	0	0	0	0
Procellariiformes	6	0	0	0	0
Pelecaniformes	2	0	0	0	0
Ciconiiformes	5	2	0	1	1
Anseriformes	13	9	1	8	9
Falconiformes	21	4	0	0	0
Galliformes	32	19	1	10	11
Gruiformes	21	9	2	1	3
Charadriiformes	11	0	0	0	0
Columbiformes	16	2	0	0	0
Psittaciformes	29	11	1	3	4
Cuculiformes	3	0	0	0	0
Strigiformes	9	0	0	0	0
Caprimulgiformes	2	0	0	0	0
Apodiformes	15	0	0	0	0
Coliiformes	0	0	0	0	0
Trogoniformes	0	0	0	0	0
Coraciiformes	2	0	0	0	0
Piciformes	9	0	0	0	0
Passeriformes	136	5	1	1	2
Total	340	62	6	24	30

* does not include the Arabian ostrich

TABLE 2. Analysis of Rare and Endangered Ciconiiformes Frequently Breeding in Captivity

Species	Year	Number of Zoos Total	Breed. Poten.	Births	Population Total	Births	Percent Captive Born
Geronticus eremita	1964	*n.d.	n.d.	3	n.d.	11+	n.d.
(Red-cheeked ibis)	1966	10	4+	3	50(28)	?	56
	1968	n.d.	n.d.	5	n.d.	10+	n.d.
	1970	14	7+		77(58)+		75

* n.d. = no data

and numbers of exhibiting institutions increasing, and the native habitat restocked. Encouraging success is also true for the cereopsis, Cuban tree duck, Laysan duck, and koloa. However, in all cases small numbers of wild-caught birds continue to be taken. Data is too limited for judgment on the Mexican duck and Aleutian Canada goose. The New Zealand brown teal, although breeding at Slimbridge and Peakirk, is not increasing. It is felt that the bulk of captive waterfowl breeding success is largely attributable to special waterfowl facilities (such as Slimbridge and Cleres) and private breeding operations. The latter may be holding numbers of these species not reported to the yearbook.

Falconiformes. Twenty-one forms (11 sp., 10 ssp.) are endangered. Four were reported in captivity, none breeding in 1964 through 1970. The southern bald eagle, doubtless represented in captivity, either could not be or was not differentiated in captivity. The California condor is represented by a single Los Angeles bird. The monkey-eating eagle was exhibited in proportionately large numbers, as follows:

1964	17 birds	1 reported pair
1965	25 "	0 " "
1966	18 "	0 " "
1967	12 "	1 " "
1968	11 "	?
1969	13 "	1 " "
1970	14 "	1 " "

The Hawaiian hawk has averaged three captive specimens each year since 1964, but infrequently as a potentially breeding pair.

TABLE 3. ANALYSIS OF RARE AND ENDANGERED ANSERIFORMES FREQUENTLY BREEDING IN CAPTIVITY

| Species | Year | Number of Zoos | | | Population | | Percent Captive Born |
		Total	Breed. Poten.	Births	Total	Births	
Branta sandvicensis	1964	15	14	2	127(127)	1+	100
(Hawaiian goose)	1966	16	9	3	93 (93)	15+	100
	1968	16	13	2	153(153)	74	100
	1970	18	16		173(172)		99
Cereopsis novae-hollandiae	1964	38	33	16	166(107)	56	64
(Cereopsis)	1966	63	51	18	246(117)	52	48
	1968	72	58	23	326(168)	74	52
	1970	74	60		341(236)		70
Dendrocygna arborea	1964	*n.d.	n.d.	3	n.d.	25	n.d.
(Cuban tree duck)	1966	23	19	6	142 (34)	22	24
	1968	36	26	5	185 (75)	37	40
	1970	37	26		183 (71)		39
Anas aucklandica chlorotis	1964	1	1	?	?	?	?
(New Zealand brown teal)	1966	4	3	1	25 (23)	23	92
	1968	5	3	1	15 (10)	6	67
	1970	3	1		12 (11)		92
Anas diazi	1964		n.d.				?
(Mexican duck)	1966		n.d.				?
	1964	5	4	n.d.	17 (10)	n.d.	59
	1970	6	5		19 (10)		53
Anas laysanensis	1964	10	10	3	107 (66)	17	62
(Laysan duck)	1966	28	25	16	174(107)	14	61
	1968	36	32	14	245(165)	64	67
	1970	48	41		292(233)		80
Anas platyrhynchos wyvilliana	1964	7	6	3	45 (34)	17	76
(Hawaiian duck)	1966	11	9	4	52 (37)	14	71
	1968	12	12	7	134 (39)	45	29
	1970	18	18		216(168)		78
Branta canadensis leucopareia	1964		*n.d.				
(Aleutian Canadian goose)	1966		n.d.				
	1968	4	2	1	8 (4)	3	50
	1970	7	4		18 (9)		50

* n.d. = no data

Galliformes. Thirty-two forms (20 sp., 12 ssp.) are reported endangered. Nineteen were exhibited in captivity, 11 with some reproductive success. Those species exhibited but not breeding were Asian forms which are extremely rare and virtually impossible to obtain, including Blyth's tragopan, western tragopan, Chinese monal, Sclater's monal, imperial pheasant, and Malaysian peacock pheasant. Also included were the greater prairie chicken and lesser prairie chicken (Table 4). Data from private breeders is no doubt lacking.

White-eared pheasant, mikado pheasant, Hume's pheasant, and palawan peacock pheasant all indicate consistent captive gains with small numbers being acquired from the wild except for the white-eared pheasant.

Elliot's pheasant, Edwards' pheasant, brown-eared pheasant, and Cabot's tragopan are essentially holding their own.

TABLE 4. ANALYSIS OF RARE AND ENDANGERED GALLIFORMES FREQUENTLY BREEDING IN CAPTIVITY

| Species | Year | Number of Zoos | | | Population | | Percent Captive Born |
		Total	Breed. Poten.	Births	Total	Births	
Tragopan caboti	1964	13	8	1	26 (10)	3	38
(Cabot's tragopan)	1966	1	1	1	8 (5)	7	63
	1968	2	2	2	13 (10)	5	77
	1970	1	1		9 (8)		89
Crossoptilon c. crossoptilon	1964	*n.d.					
(White-eared pheasant)	1966	12	8	1	37 (1)	?	3
	1968	12	5	1	18 (3)	7	17
	1970	7	6		32 (23)		72
Crossoptilon mantchuricum	1964	24	21	4	79 (62)	23	78
(Brown-eared pheasant)	1966	26	22	3	75 (29)	17	39
	1968	33	24	2	91 (49)	1	54
	1970	24	15		62 (41)		66
Lophura edwardsi	1964	23	15	2	52 (41)	2	79
(Edward's pheasant)	1966	28	16	2	82 (49)	4	60
	1968	23	15	6	66 (38)	29	58
	1970	23	14		69 (49)		70
Lophura swinhoii	1964	65	52	19	286(203)	82	71
(Swinhoe's pheasant)	1966	88	?	19	343(189)	75+	55
	1968	91	?	23	339(196)	97	55
	1970	*n.d.	n.d.		n.d.		?
Syrmaticus ellioti	1964	38	31	10	138(115)	35+	83
(Elliott's pheasant)	1966	47	37	10	169 (98)	86	58
	1968	52	39	8	216(154)	64	71
	1970	36	27		114 (81)		71
Syrmaticus h. humiae	1964	9	8	2	27 (6)	20	22
(Hume's pheasant)	1966	15	13	7	84 (42)	107	50
	1968	24	20	7	133 (83)	87	62
	1970	35	29		125 (99)		79
Syrmaticus mikado	1964	13	9	1	30 (12)	0	40
(Mikado pheasant)	1966	18	8	2	31 (4)	9	13
	1968	25	17	2	73 (37)	40	51
	1970	32	24		224(181)		81
Polyplectron emphanum	1964	*n.d.	n.d.	1	n.d.	2	n.d.
(Palawan peacock pheasant)	1966	20	16	3	78 (19)	7	24
	1968	20	16	3	80 (19)	13	24
	1970	26	14		72 (23)		32
Catreus wallichii	1964	n.d.	n.d.	4	n.d.	4+	n.d.
(Cheer pheasant)	1966	n.d.	n.d.	6	n.d.	50+	n.d.
	1968	n.d.	n.d.	5	n.d.	49	n.d.
	1970	21	16		99 (80)		81

* n.d. = no data

Cabot's tragopan indicates no additions from the wild and virtually a totally captive-born group. Elliot's pheasant, Edwards' pheasant, and brown-eared pheasant are not thought to have been imported recently and the wild-caught numbers probably reflect failure of reporting institutions to indicate origin. Swinhoe's pheasant, although remaining stable in zoos, is being managed well on Taiwan, including one re-introduction (six pairs) by the Ornamental Pheasant Trust. If one assumes some numbers of birds being released to or held by private breeders, it is probably self-supporting. Insufficient data makes comment on the Cheer pheasant difficult, although it is breeding well.

The number of institutions exhibiting these species and/or possessing breeding potential is declining or remaining stable in all cases except, Swinhoe's pheasant, Hume's pheasant, mikado pheasant, and palawan peacock pheasant. Other than the breeding program at the Arizona-Sonora Desert Museum in the early 1960s, no masked-bobwhite were reported until the 1970 census (two individuals in two zoos).

Gruiformes. Twenty-one forms (14 sp., 7 ssp.) are Red Book species. Nine are reported in IZY censuses, with three reported to have bred in captivity. Data on the whooping crane is familiar and not included here, San Antonio having the only recent zoo success. A major federal program is underway at Patuxent, Maryland. One successful hatch is reported for the Kagu. The Japanese crane (Table 5) indicates some captive breeding success, although data indicates decline in all categories.

Small numbers of Florida sandhill crane, Siberian crane, black-necked crane, horned coot, and Hawaiian gallinule were reported in captivity with no breeding. Most distressing was the dramatic increase in hooded crane numbers without known pairings, as follows:

1964	4 birds	1 breeding potential
1965	4 "	1 " "
1966	23 "	5 " "
1967	46 "	10 " "
1968	73 "	13 " "
1969	96 "	16 " "
1970	85 "	16 " "

Columbiformes. Of the 16 endangered forms, two species of pigeon (great pigeon and Mindoro imperial pigeon) were noted in captivity in small numbers with no reported breeding.

Psittaciformes. There are 29 endangered forms (10 sp., 9 ssp.). Eleven were reported in captivity, with four showing reproductive success. Table 5 shows data for the three most successful forms, all indicating upward trends in captive breeding success (hooded paradise parakee, turquoise parakeet, splendid parakeet).

TABLE 5. ANALYSIS OF RARE AND ENDANGERED GRUIFORMES, PSITTACIFORMES AND PASSERIFORMES FREQUENTLY BREEDING IN CAPTIVITY

Species	Year	Number of Zoos Total	Number of Zoos Breed. Poten.	Number of Zoos Births	Population Total	Population Births	Percent Captive Born
Grus japonensis	1964	20	13	1	50 (20)	1	40
(Japanese crane)	1966	22	13	2	60 (13)	1	22
	1968	16	8	1	34 (19)	1	56
	1970	18	8		33 (12)		36
Psephotus chrysopterygius	1964	*n.d.	n.d.	1	n.d.	?	?
dissimilis	1966	n.d.	n.d.	1	n.d.	3	?
(Hooded paradise parakeet)	1968	5	4	2	20 (14)	5	70
	1970	4	3		36 (25)		69
Neophema pulchella	1964	6	4	3	18 (17)	15+	94
(Turquoise parakeet)	1966	20	16	2	104 (50)	9	48
	1968	23	20	6	117 (66)	22	56
	1970	29	24		145 (85)		59
Neophema splendida	1964	6	3	1	13 (9)	5	69
(Splendid parakeet)	1966	8	5	6	46 (33)	60	72
	1968	11	10	3	48 (26)	30	54
	1970	14	11		101 (77)		76
Leucopsar rothschildi	1964	17	11	3	50 (5)	8+	10
(Rothschild's myna)	1966	33	15	3	111 (17)	6+	15
	1968	36	23	5	115 (39)	11	34
	1970	43	19		114 (48)		42

* n.d. = no data

The thick-billed parrot bred infrequently (three times).

Passeriformes. One-hundred-thirty-six species and subspecies of perching birds are reported as rare and endangered. Five were reported in captivity with two reported as breeding, including an isolated hatch of grey-necked rock fowl (Picathartes) and Rothschild's myna. Data for the latter is in Table 5. Captive reproductive success is increasing. Wild imports have slowed.

MAMMALS

Table 6 provides an ordinal summary for mammals. For the 19 orders of mammals, five (Monotremata, Dermoptera, Pholidota, Tubulidentata, and Hyracoidea) contain no rare and endangered forms. The remaining 14 orders contain 291 rare and endangered forms, of which 162 (55.6 percent) were represented in captivity. Endangered whales and bats were not represented. Eighty-seven forms indicated breeding in captivity (59 breeding regularly and 28 with three or less birth occurrences during the study period). This is 53.7 percent of all forms exhibited and 22.2 percent of all endangered forms.

While by comparison with birds the overall picture looks better, a detailed analysis for mammals (Perry, Bridgwater, and Horseman, 1972) indicates that only three mammals (wisent, Pere David deer, and Przewalski horse) were entirely supported by captive breeding programs, while only the mongoose lemur, Siberian tiger,

onager, Formosan sika, and addax were close to self-support. The remaining forms were either being supported by wild stock only, increasing in captivity much too slowly, or diminishing.

DISCUSSION AND SUMMARY

Among the 62 endangered bird forms reported in captivity during the period of the study, 32 are totally dependent upon wild acquisition. Six others have shown only one to three breeding occurrences. Status of the remaining 24 forms varies from the successful saving of the nene to the slow decline of Cabot's tragopan without the infusion of wild stock.

Only the Anseriformes, Galliformes, and to a lesser degree, the Psittaciformes indicate significant reproductive success.

Generally, both the number of zoos exhibiting endangered species and the number of zoos with reproductive potentials are increasing; however, only a very few specialty institutions such as the Wildfowl Trust and the Ornamental Pheasant Trust and similar private institutions are responsible for the bulk of captive-bred individuals.

The ratio of potential breeding groups to actual birth events is low and stable, while the number of zoos actually exhibiting endangered forms is increasing.

An arbitrary attempt was made to identify those species where zoo propagation gives some assurance that they could be effectively main-

TABLE 6. ORDINAL SUMMARY OF CAPTIVE STATUS FOR RARE AND ENDANGERED MAMMALS 1964-1970

Order	IUCN Forms	Forms in Captivity	Captive Breeding [1]Rarely	Frequent	Total
Monotremata	0	0	0	0	0
Marsupialia	30	4	1	3	4
Insectivora	4	1	0	0	0
Dermoptera	0	0	0	0	0
Chiroptera	2	0	0	0	0
Primates	46	32	12	8	20
Edentata	6	5	0	1	1
Pholidota	0	0	0	0	0
Lagomorpha	4	1	1	0	1
Rodentia	24	8	4	0	4
Cetacea	8	0	0	0	0
Carnivora	[2]56	31	6	13	19
Pinnipedia	11	6	0	0	0
Tubulidentata	0	0	0	0	0
Proboscidea	1	1	0	0	0
Hyracoidea	0	0	0	0	0
Sirenia	5	5	0	0	0
Perissodactyla	17	14	1	11	12
Artiodactyla	77	44	3	23	26
Total	291	162	28	59	87

[1] Less than 3 births reported
[2] Includes Bengal tiger

tained without wild infusions. The following criteria were applied to the 62 captive bird forms: 1) current captive population of at least 125; 2) 50 percent of total population captive born; and 3) present at 20 institutions with breeding potential.

Using these criteria, only Swinhoe's pheasant, mikado pheasant, Hume's pheasant, Elliott's pheasant, nene, cereopsis, Laysan duck, Hawaiian duck, Cuban tree duck, and turquoise parakeet qualify.

These criteria are no doubt minimal, and if breeding programs are not quickly effected concentrating upon captive survival, and if the expenditure of effort, time, money, and technical resources is not provided by joint effort and at the expense of an "animals for exhibit only" philosophy, then zoos may be in danger of failing in their preservation and propagation objectives.

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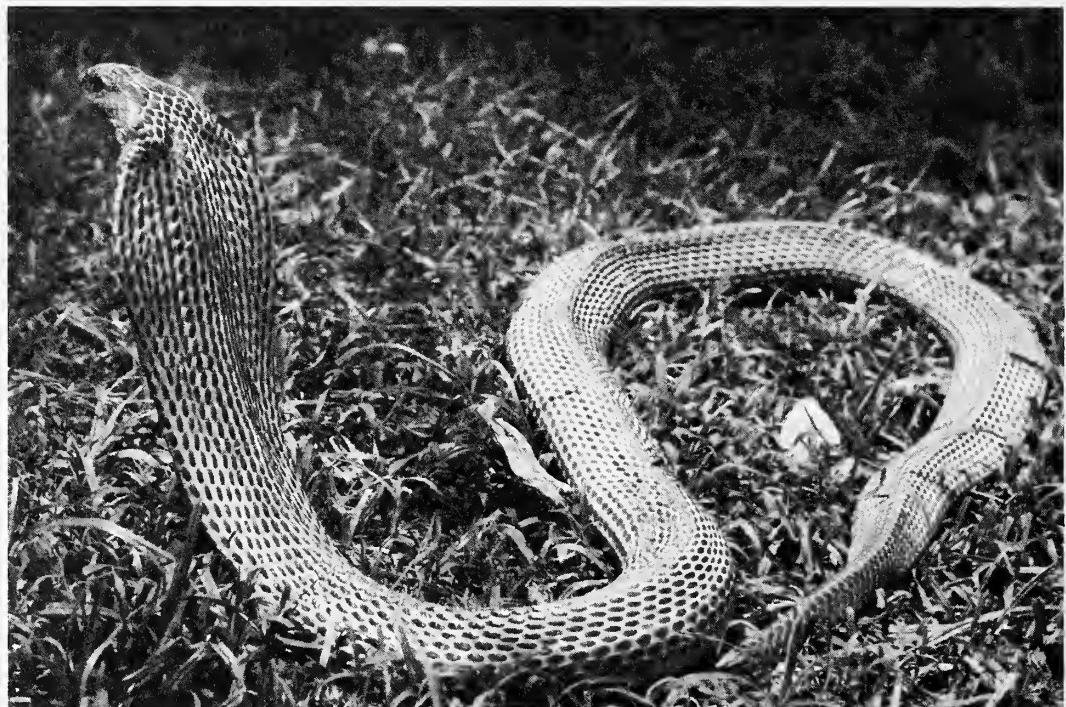
PLATE I.   Philippine Cobra, *Naja naja philippinensis.*

9

Venom Yields of the Philippine Cobra, *Naja naja philippinensis*

(Plate I; Text-figure 1; Tables I-II)

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Data on venom yields of 150 cobras (*Naja naja philippinensis*) gave an overall average venom yield per cobra per extraction (AVY/C/E) of 0.33 ml or 70.14 mg and an overall average total lifetime venom yield per cobra (ATLVY/C) of 2.23 ml or 527.77 mg for the fresh and corresponding dried venom, respectively.

The ATLVY/C were greater at schedule every 14 days than those at schedule every 7, 21, or 28 days. The all-male groups gave bigger yields than the corresponding all-female groups. The AVY/C/E in the former increased as the intervals between extractions lengthened. In the latter the reverse was observed.

The solid content of the venom does not appear to be affected by either sex, time of the year, or schedule of collection, but the serial records of extractions show a tendency for this to gradually decline with time. Venom extraction at close intervals resulted in a marked decline of the solid content, the rate of reduction becoming more marked in direct proportion to frequency of extraction.

The data on AVY/C/E of several groups indicate a general tendency for this to decline with time.

Introduction

In view of the absence of venom suppliers in the Philippines, it is essential that the Serum and Vaccine Laboratories (SVL) undertake the production of the venom to carry on its antivenin preparation program. For this purpose, a serpentarium is maintained for cobras of the species *Naja naja philippinensis*, and sufficient number of snakes are kept to ensure an adequate and uninterrupted supply of good quality venom. The number of snakes is replenished with cobras caught during field collection trips undertaken every three or four months.

To provide a basis for a realistic estimate of the number of cobras needed to supply an adequate amount of the venom to meet the requirements of the laboratory for antivenin production, records of venom yields are necessary. The present study was conducted for this purpose as well as to enable us to work out a schedule of venom collection which will assure maximum yields with the facilities available. Experiments were designed to furnish information on the possible effects of factors like sex and schedule or frequency of extraction on venom yields. The possibility of trends in yields with time was also investigated.

To the clinicians, data on venom yields may serve as aids in making an estimate of the amounts of the specific antivenin which may be required to neutralize the venom injected in a bite.

Oliver (1944) states that "the quantity of venom secreted in the act of biting varies according to the species, the size, age, and the condition of the snake at the time of the bite. In general, the larger the snake, the greater the quantity of venom injected, though there are many exceptions to this generality. The amount of venom injected depends on the time interval since the last bite, the venom glands usually requiring approximately two weeks to regain their maximum capacity of venom. In a normal bite, a snake does not expel its full quantity of venom, but only a small portion and is still capable of inflicting a fatal bite. Evidence indicates that an enraged snake injects a greater quantity of venom than one which has not been angered prior to biting. The amount of venom released during a spontaneous bite is greater than that obtained by investigators through 'milking' or forced expulsion of the venom."

He gives the following figures on the approximate amounts of dry venom obtained at a single

extraction from common poisonous snakes:

A. North American Species	Mg.
Copperhead	45– 65
Water moccasin	90–150
Timber rattler	40– 90
Texas rattler	120–300
Florida rattler	240–450

B. South and Central American Species

Tropical rattlesnake	60–150
Fer-de-lance	80–160
Bushmmaster	300–500

C. Indian Species

Asiatic cobra	250–350
Russell's viper	200–300

D. African Species

Mamba	50– 80
Puff adder	70–120

E. Australian Species

Tiger snake	35– 50
Death adder	60– 80

Christensen (1955), referring to the works of Grasset, Zoutendyk, and Schaafsma, and Grasset and Schaafsma gave the following records of yields of venom of various snakes:

Snake	Average Yield	Limit of Individual Yields	Remarks
N. flava	0.12 g (128 specimens)	Maximum–0.25 g	
D. angusticeps	0.1 g		
N. haje	0.72 g (1 snake)		Length–7′3″ An enormous yield
S. haemachates	0.42 ml (0.1 g) (over 150 specimens)	0.1 ml–1.8 ml (0.033 g–0.242 g)	Solid content just under 25%
B. arientans	0.18 g first milking 0.07 g five days in captivity 0.1 g three weeks in captivity	Maximum–0.75 g	
D. typus	0.015 g average yield from pair of glands		41% W/W solid content; (obtained direct from gland)

Other data on venom yields mentioned by Christensen include:

Fifteen full grown puff adders gave an average yield of 0.67 ml (0.186 g).

The following table gives data on venom obtained from different snakes in his laboratory:

Species	Size		Yields*			Percentage** Solid		
	No.	Cm.	Min.	Max.	Ave.	Min.	Max.	Ave.
S. haemachates	10	90–120	0.11	0.72	0.35	17.5	28.3	23.9
N. flava	1	150			0.11			27.1
N. haje	1	150			0.12			33.5
C. rhombeatus	9	45– 75	0.07	0.75	0.34	19.3	28.1	23.6
B. cornuta	5	32– 35	0.008	0.087	0.045	24.5	36.4	31.1
B. caudalis	1	60			0.085			27.6
B. gabonica	1	113			1.90			26.4

* Venom from B. cornuta and B. caudalis in g, the others in ml.

** W/W for B. cornuta and B. caudalis venom W/V for others.

Christensen states that the quantity, composition, and toxicity of the venom of a given species may vary considerably. He mentions that age, state of health, climate, and even the "mood" of the snake may affect the character and quantity of venom. Conant (1952) states that the snake has some control over the amount of venom injected in a bite. Minton (1957) gives the average amount of venom delivered in a bite by the following venomous species:

A. Elapidae	Mg.
North American coral snake (*Micrurus fulvius*)	3– 5
Blue krait (*Bungarus candidus*)	5– 10
Tiger snake (*Notechis scutatus*)	35– 45
Indian cobra (*Naja naja*)	175–250
Mamba (*Dendroaspis angusticeps*)	75–100
B. Viperidae	
African puff adder (*Bitis arientans*)	160–200
Russell's viper (*Vipera russelli*)	150–250
C. Crotalidae	
Fer-de-lance (*Bothrops atrox*)	80–160
Bushmaster (*Lachesis muta*)	300–500
Western diamondback rattlesnake (*Crotalus atrox*)	200–300

Deoras (1966) mentions environment, sex, seasonality, and frequency of milking as possible factors affecting venom yields, and in his studies showed that cobras and kraits produced more venom when kept in a farm under conditions simulating that of their natural habitat than when kept in separate cages in a room. The vipers however, produced more venom when kept in the room.

Materials and Methods

A total of 150 cobras, *Naja naja philippinensis*, 69 males and 81 females, with an overall average length of 47.7 inches collected in the province of Camarines Sur, Luzon Island, were used to provide the data presented in this study. All were apparently healthy and freshly caught at the beginning of each experiment except as indicated. Distribution of specimens into groups for comparison was done in a completely random manner from batches comprised of specimens approximately of same lengths. The care and management of the snakes and the method of venom collection employed, using a beaker with rubber diaphragm, have been previously described (Salafranca, 1967). The freshly collected venom was spread thinly in petri dishes and placed inside a dessicator over calcium chloride. The dessicator was sealed and evacuated using a high vacuum pump. It was kept in the cold room (4°-10°C) until the dried crystalline venom could be easily peeled off the glass. This required one to three or more days depending upon the thickness of the layer or the amount of the venom and the condition of the calcium chloride. The schedule of venom collections for any given experiment was observed until all the snakes had died.

EXPERIMENT I. Venom was collected from a group of 29 cobras, 7 males and 22 females with an average length of 48 inches, every 14 days. The pooled amount was recorded for each collection schedule.

The average venom yield per cobra per extraction (AVY/C/E) was computed by dividing the total amount collected by the number of snakes involved at each extraction.

Following the last collection, the overall AVY/C/E and median were computed.

The average total lifetime venom yields per cobra (ATLVY/C) was computed by dividing the total amount of venom collected from each schedule group by the number of snakes involved in the group.

The percent solid was computed by dividing the weight of the dry venom by the weight of the corresponding "wet" venom.

EXPERIMENT II. Thirty cobras with an average length of 48.9 inches, 15 males and 15 females, were selected from a catch of 37 specimens on the basis of similarities in sizes. Each sex group was randomly divided into three equal groups and each group of males paired at random with a group of females. Each of the three resulting groups was assigned to one of three schedules of venom collection, as follows:

Group	Schedule of Venom Collection
IIa	Every 7 days
IIb	Every 14 days
IIc	Every 28 days

EXPERIMENT III. Thirty-eight cobras with an average length of 48.9 inches, 19 males and 19 females, were distributed at random into four groups and their venom collected as indicated below:

Group	Number and Sex	Scheduel of Venom Collection
IIIa	10 females	Every 14 days
IIIb	10 males	Every 14 days
IIIc	9 females	Every 28 days
IIId	9 males	Every 28 days

EXPERIMENT IV. Fifty cobras of average length, 46.6 inches, 25 males and 25 females,

were distributed into six groups and their venom collected according to the schedule indicated below:

Group	Number and Sex	Scheduel of Venom Collection
IVa	8 males	Every 14 days
IVb	8 females	Every 14 days
IVc	9 males	Every 21 days
IVd	9 females	Every 21 days
IVe	8 males	Every 28 days
IVf	8 females	Every 28 days

EXPERIMENT V. To determine the capacity for venom production of individual cobras, the following experiment was performed:

Three male cobras were selected and subjected to repeated venom extractions at regular intervals during a whole working day according to the following schedule:

Cobra Number	Length (inches)	Schedule of Venom Collection
1	45	Every 2 hours
2	43.5	Every hour
3	39	Every half-hour

Cobra no. 1 and cobra no. 2 were obtained in our regular periodic hunt and had been in the laboratory serpentarium 22 days at the time of this experiment. Cobra no. 3 was a specimen caught within the laboratory premises two days before this experiment.

A 50-ml beaker of known weight was assigned to each snake. The venom was extracted as described previously (Salafranca, 1967). Due to the anticipated difficulty of getting accurate volumetric measurements, the weight of the venom collections were determined instead. The initial amount collected was rated 100% and the subsequent collections as percentages of the initial collection.

The data on the serial amounts of venom collected from certain groups in experiments one to four were plotted in a graph to determine possible trends with time.

RESULTS AND DISCUSSION

The results of Experiments I to IV are summarized in Table I. A comparison of the ATLVY/C in three comparable groups of cobras, IIa, IIb, and IIc of Experiment II, subjected to 7, 14, and 28-day schedules, respectively; in four comparable groups, IIIa and IIIb,

and IIIc and IIId, of Experiment III, subjected to 14 and 28-day schedule, respectively; and in six comparable groups, IVa and IVb, IVc and IVd, and IVe and IVf of Experiment IV, on 14, 21, and 28-day schedules, respectively, shows that, in each case, the average was greater in the group or groups on the every 14-day schedule of extraction. In Experiment II, using the dry venom data, the ATLVY/C on the 14-day schedule (IIb) was 16% and 26% (or 12% and 11% on the "wet" data) greater than those on 7-day and 28-day schedules (IIa and IIc), respectively. In Experiment III, the all-female and all-male groups on the 14-day schedule (IIIa and IIIb) averaged 35% and 37% (or 55% and 15% on the "wet" data) more venom than the corresponding all-female and all-male groups on the 28-day schedule (IIIc and IIId). The data for the all-male and all-female groups on the 14-day schedule in Experiment IV (IVa and IVb) show 15% and 9% and 18% and 25% (or 26% and 17% and 24% and 45% on the "wet" data) greater ATLVY/C than those in the corresponding all-male and all-female groups on the 21 and 28-day schedules (IVc and IVd and IVe and IVf), respectively.

From the data on the dry venom yields for comparable all-male and all-female groups, it will be observed that the former consistently gave greater ATLVY/C. In Experiment III, the all-male groups on the 14 and 28-day schedules (IIIb and IIId) gave 32% and 73% (or 50% and 102% on the "wet" data) more venom than the corresponding all-female groups (IIIa and IIIc), while in Experiment IV the all-male groups on 14, 21, and 28-day schedules (IVa, IVc and IVe) gave 87%, 93%, and 114% (or 102%, 100%, and 149% on the "wet" data) more than the corresponding all-female groups (IVb, IVd, and IVf).

The AVY/C/E on the other hand, in comparable groups, was observed to increase with the increase in intervals between extraction both in the mixed sex groups as well as in all-male groups. In Experiment II, we have 58.14 mg, 77.6 mg, and 101.73 mg for 7, 14, and 28-day schedules, and in Experiment IV, 72.73 mg, 75.75 mg, and 95.71 mg for the all-male groups on 14, 21, and 28-day schedules, respectively. The increase in AVY/C/E with the increase in intervals between extraction appear logical, since the gland is given correspondingly more time to recover its full capacity. With the all-female groups, however, the order is reversed; that is, the AVY/C/E decreases with increasing intervals. Thus we have for the all-female groups in Experiment III, 63.66 mg and 57.1 mg for the 14 and 28-day schedules, and in Experiment IV, 40.74 mg, 40.46 mg, and 35.66 mg for the 14,

Experiment Number	Period of Handling	Schedule of Venom Extraction (interval in days)	No. of Snakes M	No. of Snakes F	Venom Yields AVY/C/E* Wet (ml)	AVY/C/E* Dry (mg)	ATLVY/C* Wet (ml)	ATLVY/C* Dry (mg)	Average % Solid
I	8/15/63 to 3/20/64	14	7	22	a – 0.20 / m – 0.18	a – 53.95 / m – 58.39	1.94	520.16	a – 26.76 / m – 27.65
IIa	2/4/64 to 4/27/64	7	5	5	a – 0.21 / m – 0.20	a – 58.14 / m – 53.37	2.24	633.39	a – 29.54 / m – 25.50
IIb	2/4/64 to 7/7/64	14	5	5	a – 0.29 / m – 0.28	a – 77.60 / m – 75.09	2.60	709.42	a – 26.30 / m – 28.50
IIc	2/4/64 to 7/21/64	28	5	5	a – 0.33 / m – 0.33	a – 101.73 / m – 97.70	2.07	641.28	a – 29.25 / m – 29.80
IIIa	5/16/64 to 10/18/64	14	0	10	a – 0.28 / m – 0.28	a – 63.66 / m – 60.41	2.33	562.23	a – 22.66 / m – 21.00
IIIb	5/16/64 to 11/17/64	14	10	0	a – 0.36 / m – 0.34	a – 92.86 / m – 78.21	3.08	844.41	a – 24.69 / m – 23.00
IIIc	5/16/64 to 11/5/64	28	0	9	a – 0.29 / m – 0.30	a – 57.10 / m – 53.80	1.72	363.29	a – 27.72 / m – 17.42
IIId	5/16/64 to 11/12/64	28	9	0	a – 0.52 / m – 0.55	a – 115.96 / m – 109.45	2.97	733.91	a – 21.20 / m – 19.00
IVa	8/25/64 to 1/15/65	14	8	0	a – 0.39 / m – 0.40	a – 72.73 / m – 77.70	2.92	613.71	a – 17.45 / m – 19.00
IVb	8/25/64 to 1/15/65	14	0	8	a – 0.24 / m – 0.23	a – 40.74 / m – 41.32	1.56	303.52	a – 16.86 / m – 18.00
IVc	8/25/64 to 1/27/65	21	9	0	a – 0.41 / m – 0.41	a – 75.75 / m – 67.43	2.53	487.89	a – 17.56 / m – 15.75
IVd	8/25/64 to 1/29/65	21	0	9	a – 0.24 / m – 0.21	a – 40.46 / m – 37.05	1.31	244.13	a – 17.15 / m – 16.40
IVe	8/25/64 to 2/16/65	28	8	0	a – 0.54 / m – 0.53	a – 95.71 / m – 106.35	2.68	521.74	a – 17.85 / m – 19.00
IVf	8/25/64 to 2/14/65	28	0	8	a – 0.26 / m – 0.21	a – 35.66 / m – 29.89	1.25	209.73	a – 15.07 / m – 15.00
Totals			66	81					
Overall Averages					0.33	70.17	2.23	527.77	22.14

AVY/C/E – Average venom yield per cobra per extraction
ATLVY/C – Average total lifetime venom yield per cobra

a – Average
m – Median

TABLE I. SUMMARY OF RESULTS, EXPERIMENTS I-IV.

Cobra I.D.	Schedule and Record of Extractions* (Wet – mg)	Total Amount Collected (Dry – mg)	% Solid **
No. – 1 Sex – M Length – 45"	Every 2 hours 287.25 (100%) 166.84 (58%) 78.38 (27%) 94.68 (33%) 173.32 (60%)	81.4	10%
No. – 2 Sex – M Length – 43.5"	Every hour 200.94 (100%) 102.25 (51%) 129.46 (64%) 144.31 (72%) 86.29 (43%) 71.60 (36%)	53.27	7%
No. – 3 Sex – M Length – 39"	Every half hour 480.72 (100%) 433.02 (90%) 392.47 (82%) 203.24 (42%) 163.67 (34%) 228.29 (47%) 125.47 (26%) 161.25 (34%) 152.06 (32%) 113.03 (24%) 142.14 (20%) 73.63 (15%) 98.15 (20%) 74.70 (16%) 50.25 (10%)	122.13	4%

* Amount of venom given are in the chronological order of extractions. Initial extractions are rated 100% and subsequent extractions as percentages of the initial amount.

** Obtained by determining the relation of total dry weight to total weight of corresponding wet venom.

TABLE II. SUMMARY OF RESULTS, EXPERIMENT V.
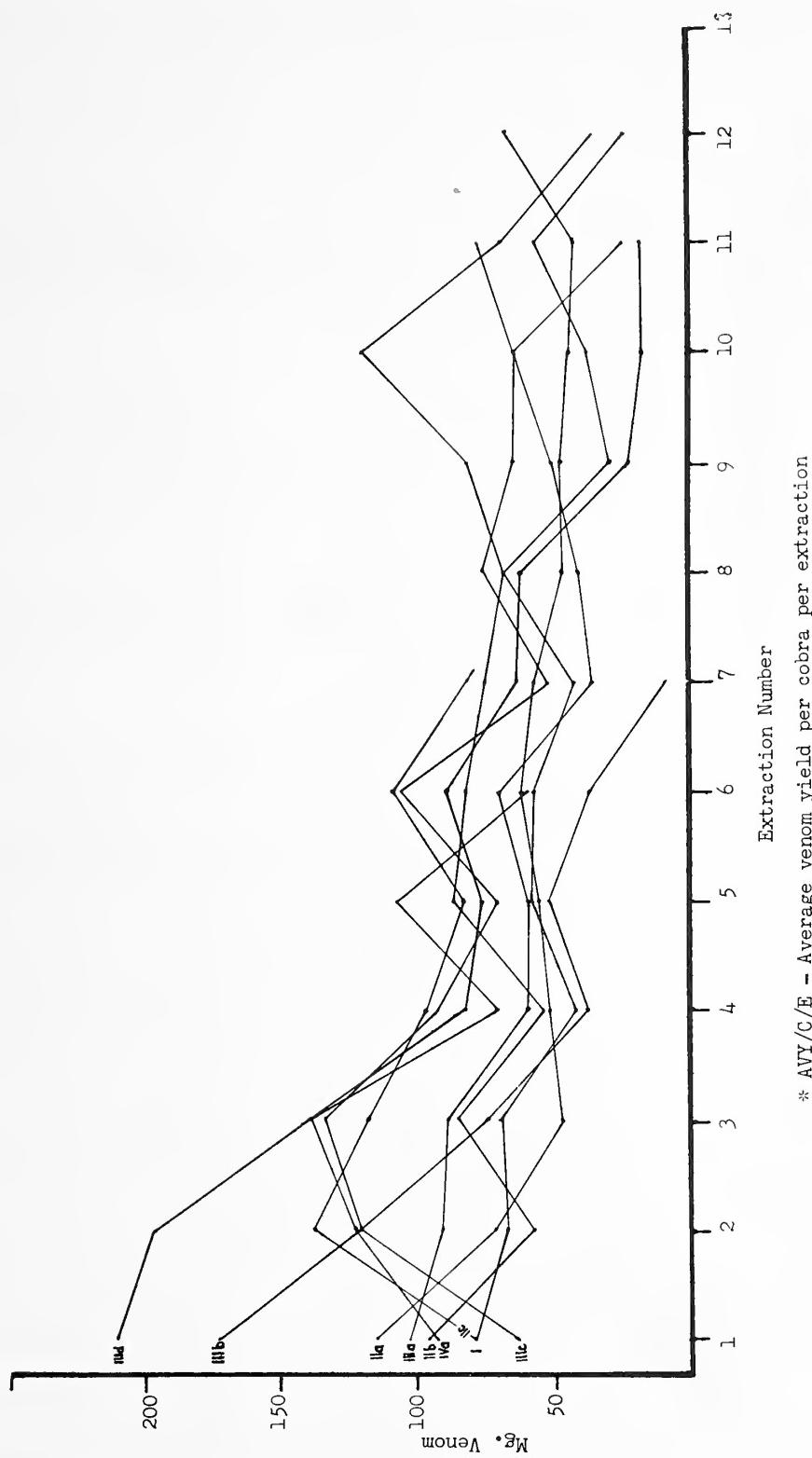
TEXT-FIGURE 1. Graphic presentation of AVY/C/E* of indicated groups in Experiments I, II, III, and IV.

21, and 28-day schedules, respectively. The observation that the males give more than the females, however, is as true when we consider the data on ATLVY/C as the data on the AVY/C/E in comparable all-male and all-female groups.

The data on average percent solids of the venom collected from the various groups in Experiments I, II, III, and IV do not give indications that this may be affected by either sex, season, (period of the year when the experiment was undertaken), or the schedule of venom extraction observed. The records of the serial extraction of the majority of individual groups, however, show a tendency for this to decline gradually as the number of extractions increased.

The results of Experiment V are summarized in Table II. It will be observed that the amounts obtained generally decreased in the order of the chronology of extraction. The amounts obtained from cobra no. 3, notwithstanding its smaller size and greater frequency of extraction (every half-hour), are greater. This may be explained by the fact, as pointed to above, that this specimen at the time of the experiment was only two days in captivity and therefore more vigorous than the other two which have been in captivity 22 days at the time of this experiment. The percent solids of the combined extractions from each snake decreased as the frequency of extraction increased, from 10% for the extractions every two hours to 4% for those at every half-hour. The percent solids of 10%, 7%, and 4% obtained for the pooled collections from snakes 1, 2, and 3, respectively, are much lower than the overall average of 22.14 (15.07% to 29.54%) percent solids from the yields in Experiments I to IV (Table I). Apparently, repeated extractions at the close intervals of every two hours, hourly, and every half-hour, results in marked dilution of the venom, the dilution increasing in that order.

Graphical examination of the chronological AVY/C/E of several groups included in Experiments I, II, III, and IV (Text-figure 1) indicates a general tendency for yields to decrease with time. In some groups, the drop in yield from the first collection to the second or third is more marked than in others. In a few, there is a rise from the first extractions to the second or third, and from then on a general but gradual tendency to diminish with occasional peaks which are generally lower than the maximum noted for that particular group.

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NEWS AND NOTES

Preliminary Report: Status Investigations of Morelet's Crocodile in Mexico

FIELD INVESTIGATIONS in the State of Veracruz, Mexico, were carried out in early August, 1971, to examine the status of several populations of Morelet's crocodile and to obtain basic information on their natural history and ecology.

Habitat surveys, population estimates, and behavioral observations, by both day and night, were undertaken in two locations, nr. Alvarado in the mouth of the Papaloapan River and Alvarado Lagoon, and in Lake Catemaco in the Tuxtlas Range. Two field days and one night were spent in the former locality, five days and six nights at the latter.

In addition to actual field observations of the crocodiles themselves a special effort was made to contact and become friendly with locals in each area who were reputed to be knowledgeable about crocodiles and/or involved in the crocodile hide industry.

For convenience, the two study areas will be treated separately.

LAGO DE CATEMACO

Habitat. Lake Catemaco is a freshwater lake of some 50 square miles located in the Sierra de las Tuxtlas at approximately 1,100 feet altitude. One major river, the Quetzaloapan, enters the lake. This forms a marsh of considerable extent where it runs into the lake on the eastern side. Several islands of several acres each dot the lake and there are several shallow embayments scattered along the shore line, the largest of which is the *Arroyo Agrio* on the north shore east of the town of Catemaco at the Coyame bottling plant. At the current time the crocodile populations are concentrated in the *Arroyo Agrio*, the *Arroyo* (Quetzaloapan River marsh), and to a lesser extent in the other embayments and on the islands. The shores of the lake still support considerable tropical vegetation though extensive clearing and other modifications of the natural cover is underway at diverse points around the lake. For the most part this habitat alteration does not involve the immediate lake shore except in a few points where human habitations are being constructed near the water's edge.

The lake is drained along the southwest end by a river, which is dammed where it crosses the major highway, Mex. Hwy. 180. Crocodiles currently exist immediately below the dam in a small impoundment and, by report, throughout the river.

Estimated Population. Accurate census of the population by night was hindered by clear skies and a full moon. Despite this handicap crocodiles were seen frequently in the *Arroyo Agrio* and the *Arroyo* and at scattered localities throughout the lake. These observations are too scattered and erratic to support more than a crude estimate of the population's size; a conservative estimate might be 200 crocodiles (all sizes) in the lake. The estimates of local people ranged from several hundred to a thousand. This larger figure is rather unlikely, but cannot be refuted at this time.

At the time of this visitation, the young of the year had not yet appeared and three nesting females were located. The nests are mounds of vegetation located on the shore in densely vegetated areas and are placed from 10 to 40 feet from the water's edge. They are highly reminiscent of the nests of the American alligator.

The lake houses at least two individuals which approach eight to nine feet in length. One of these frequents the Arroyo Agrio and the other a spit of rock backed by a small marsh south of and adjacent to the outfall of the Quetzaloapan River.

Hunting Pressure. At the present time, hunting in the lake is minimal. Seven individuals, including a five-foot specimen, are held in captivity in a restaurant, "Las Olas", on the lake shore in Catemaco. They are well fed and cared for, and the owner of the restaurant has a positive attitude toward the fauna and discourages the fishermen from molesting the wild population.

All the local fishermen questioned expressed a dismay at the difficulty of marketing hides and claimed they no longer seek crocodiles though they may take one occasionally as a target of opportunity. Skins, they claim, must be sent to San Luis Potosi or Laredo for sale.

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One *lagartero* of considerable repute lives on the lake. It was not possible to determine the extent of this individual's activities on this visit, though it was claimed he was not hunting at this time. This individual displays a commendable ecological insight in his pursuit and makes every effort to protect crocodile nests from disturbance by other humans in the area. Guides and fishermen in the area were initially reluctant to take me to nests until reassured repeatedly that I only wanted to photograph the nests and would not disturb them.

The general consensus in the area was that the primary drain on the lake's population currently is from visiting "sportsmen" who pay the local boatmen to take them out hunting. Most boatmen are reluctant to do this, but are susceptible to economic coercion.

Prospectus. Prospects for the crocodile population in Lake Catemaco appear good if the pointless, random depradations of tourist sportsmen can be controlled, and if development activities around the lake can be regulated to provide a buffer strip of natural area immediately along the lake edge, particularly in the shallow bays and arroyos.

An American ornithologist, William Schaldach, currently lives in Catemaco and is anxious to provide support for conservation activities in the area. He has already been successful in halting the slaughter of water birds along the lake by emphasizing their value as tourist attractions and hopes to extend this coverage to the crocodiles and turtles in the lake as well. His property on the lake shore provides a primitive, but convenient, research station for visiting biologists. It would appear that Catemaco might be an excellent locale to establish a reserve for Morelet's crocodile if such an undertaking is feasible.

ALVARADO

Alvarado is located on a large lagoon and an extensive salt-to-brackish marsh system which provides a great expanse of habitat for crocodiles of two species, *Crocodylus moreletii,* confined to the fresh-water portions, and *C. acutus,* in the salt and brackish areas. Several rivers empty into this lagoon, but only the Papaloapan was examined. The town of Alvarado houses a market place where many species of aquatic reptiles can be purchased for food and where crocodiles of both species have always been available.

This market had been visited in spring, 1970, and crocodiles were readily available. On the current visit the situation was considerably changed. Few fishermen would admit to hunting crocodiles and none of the animals were available for purchase, at least they were not displayed in the open. All fishermen claimed the topic was a "delicate" one and stated that hides were difficult to sell. Some hide traffic still exists, the chief port of exit being San Luis Potosi, but most fishermen claim that it is no longer a major activity.

One night visit was made in the lagoon and up into the Papaloapan River. Five crocodiles were observed but could not be approached for positive identification due to bright moonlight. Two of the crocodiles, both four feet to six feet estimated length, were observed in the lagoon itself and were presumably *C. acutus*. The remaining three specimens, in the two to three foot size range, were observed in the predominantly fresh-water situation in the Papaloapan River. My guide claimed these to be *pardos,* or *C. moreletii,* and said the specific area we were in contained only *pardos*. This identification, of course, should be considered tentative, but the habitat relationship was proper for *C. moreletii*.

In view of the uncertainties inherent in crocodile hunting and the unfavorable light levels encountered at this visit, I feel that the sighting of five crocodiles in five hours of boat-time is an encouraging development. Certainly the crocodile populations in this extensive marsh system have not been completely hunted out and may prove to be healthy enough to achieve a resurgence given effective protection.

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The senior author will be furnished first galley proofs, edited manuscript, and first illustration proofs, which must all be returned to the editor within three weeks of the date on which it was mailed to the author. Papers for which proofs are not returned within that time will be deemed without printing or editing error and will be scheduled for publication. Changes made after that time will be charged to the author. Author should check proofs against the edited manuscript and corrections should be marked on the proofs. The edited manuscript should not be marked. Galley proofs will be sent to additional authors if requested. Original illustrative material will be returned to the author after publication.

An abstract of no more than 200 words should accompany the paper.

Fifty reprints will be furnished the senior author free of charge. Additional reprints may be ordered; forms will be sent with page proofs.

Contents

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10

Daily Activity Patterns and Effects of Environmental Conditions on the Behavior of the Yellowhead Jawfish, *Opistognathus aurifrons* with Notes on its Ecology.

(Plates I-V; Text-figures 1-21; Tables 1-4)

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Quantitative observations of the behavior of *Opistognathus aurifrons* were made at Bimini, Bahamas, for a period of 25 days using an underwater television system. Various activities of this species were described. The activity of feeding was constant during the day, but was reduced during periods of low light intensity and high current speed. The activities of "digging," "chasing," and "arching" varied diurnally, and "digging" and "arching" varied with the current speed.

Light was a controlling factor of the uncovering of the burrow in the morning. Light and interspecific relations determined the time of closing the burrow in the evening. Various burrow-oriented activities showed a peak during dawn or dusk periods. The ranges and territories of individuals were determined. The vertical range varies diurnally. The relationship of *O. aurifrons* to other jawfishes and convergent species of reef fishes was examined.

INTRODUCTION

THE YELLOWHEAD JAWFISH, *Opistognathus aurifrons* (Jordan and Thompson), (Plate I, fig. 1), occurs in the Florida Keys, the Bahamas, and the West Indies (Böhlke and Chaplin, 1968). The species (maximum standard length approximately 94 mm) is found in colonies in areas of calcareous sand substrate near coral outcrops. Its known depth distribution is from 3 to 50 m.

Opistognathus aurifrons was originally described as *Gnathypops aurifrons* by Jordan and Thompson (1905) from a specimen collected at Dry Tortugas, Florida, and placed into the genus *Opisthognathus* when these two genera were shown to be synonymous (Meek and Hildebrand, 1928). Briggs (1961) pointed out that the name *Opistognathus* was used by Cuvier (1817) and that the variation *Opisthognathus* was introduced by Cuvier and Valenciennes (1836) and followed in subsequent works.

Longley and Hildebrand (1941) included some general information about *O. aurifrons*. They described the burrow as "perhaps 300-500 mm deep" and "enlarged below, the shape of the terminal chamber being largely fixed by the arrangement of the larger bits of dead coral by which it is surrounded." One fish was observed in the midst of constructing its burrow and its behavior described. The general feeding behavior was described and its food characterized as planktonic.

Böhlke and Thomas (1961) redescribed *O. aurifrons* and placed in its synonymy *Gnathypops bermudezi* Howell Rivero. They dealt with geographic variation in certain characters of specimens from Florida, the Bahamas, the Virgin Islands, and Cuba. They found Bahamian specimens generally to have black pigment spots on the chin and under the gill membrane at the isthmus, a curved dark line beneath the maxillary and the preopercle, and the branchiostegals edged with duskiness. Specimens from Florida lacked all of these markings, and material collected in the Virgin Island population was found to be intermediate to the Florida and Bahama populations in the number of branched dorsal, anal, and pectoral-fin rays, length of the lateral

line, number of lower gill rakers, and the number of canine teeth. An increase in the number of lower gill rakers with increasing standard length was also found.

Böhlke (1967) reported that one specimen of a large series taken in the Florida Keys (UMML 18904) showed all the black head markings characteristic of Bahamian individuals.

Böhlke and Thomas (1961) also dealt with the tear drop shape of the pupil of *O. aurifrons.* The long axis of the pupil is oriented horizontally when the fish is in a normal vertical "floating" or hovering position, and they thought that this, plus the position of the eyes on the head, allows binocular vision horizontally when the fish is in its normal hovering orientation.

Böhlke and Thomas (1961) reported a depth distribution of from 3 to 30 m. Böhlke (1967) modified this distribution to 3 to 36 m for Bahamian specimens and to 41 m for Florida specimens. The writer has observed *O. aurifrons* at Long Reef, Florida, to a depth of 50 m. Specimens in the Florida Keys are found most often on the seaward side of the outer reefs in depths greater than 7 m. In Bimini, Bahamas, *O. aurifrons* is found on the seaward (west) side of the islands.

Randall (1967a) examined the stomach contents of 16 specimens from the Virgin Islands and determined their food consisted of 85 percent copepods, 9.4 percent shrimp larvae, and small percentages of fish eggs, siphonophores, barnacle larvae, polychaetes, and unidentified animal remains. He also states *O. aurifrons* is diurnal and "covers the entrance to its burrow for the night by backing in with a large stone in its jaws."

Leong (1967) gave a general account of the breeding and territorial behavior in the yellow-head jawish. She dealt with fish from the Florida region in aquaria and described both males and females as being territorial, even during pair formation and breeding. Paired fish were allowed to enter one another's territories and burrows; sexual dimorphism in behavior was seen for paired fish. Both fish frequented a third burrow and the male fish led the female to this burrow by performing a lateral display action. Spawning occurred in the burrow and the male orally incubated the eggs. The eggs could be laid down inside the burrow to allow the male to eat. A brooding male was allowed to enter the female's burrow, but the female was not permitted to enter the brooding male's burrow.

There are differences in color pattern of Bahamian and Floridian specimens aside from the already mentioned head markings. Böhlke and Thomas (1961) provided an excellent description of life colors for Florida specimens.

Bahamian specimens as illustrated in Böhlke and Chaplin (1968: 489) and in Plate I, fig. 1 are paler than individuals from Florida. The yellow found on the anterior portion of the body of Florida specimens is much less intense in those from the Bahamas and is practically non-existent in specimens maintained in aquaria for a few weeks. The various dark markings on the head of Bahama individuals, with the exception of the spots on the lower jaw, are normally hidden by various bones and folds of skin. These markings are clearly exposed during various intraspecific activities and are probably important in such activities. Also present on many Bahamian individuals is what may be termed an "eyebar," a broad faint, dark band running dorsally between the eyes and ventrally onto the tip of the lower jaw. Unfortunately this band is seldom visible in preserved material although it is obvious in life.

These important morphologic differences between Bahamian and Florida populations indicate that consistent differences may well exist also in behavior. Therefore this study was devoted when feasible only to the Bahamian population. Some supplementary information was obtained for the Florida population and such is noted in the text.

Since it often is displayed in home and public marine aquaria, much popular literature also exists on *O. aurifrons.* Some of the more noteworthy references to this literature include Ray (1968), Van Doorne (1969), and Kristensen (1965).

MATERIAL AND METHODS

Behavioral observations were made through the use of the University of Miami Rosenstiel School of Marine and Atmospheric Science video-acoustic installation at Bimini, Bahamas. The underwater television system (UTV) consists of a closed circuit television camera and associated hydrophones (Plate II, fig. 2) situated 1.5 kilometers off the west coast of North Bimini at a depth of 20 m with cables leading to a monitor room (Plate II, fig. 3) at the Lerner Marine Laboratory of the American Museum of Natural History. Details of the system can be found in Myrberg et al. (1969).

Daily activity of individual fish was monitored for 30-minute periods every two hours between 7 AM and 7 PM at the start of the study. This schedule was modified in relation to changing day length in order to retain the same relation of periods to total day length as was present at the onset of the activity measurements. The occurrence of specific activities was recorded by marks made upon an Esterline Angus travelling chart recorder and their frequency and/or duration determined from these

records. Measurements of the environmental parameters of current speed, current direction, and water temperature were taken at the same time as the behavioral measurements for possible correlation.

A Hydro Products model 460 current meter, with a useful range of 0.05 to 7.0 knots and an accuracy of ±3 percent of the reading was used with a model 451 current speed readout module located in the monitor room at the Lerner Marine Laboratory. The rotational speed of the current meter could also be monitored visually on the UTV as a check on the readout system. The direction of the current was determined by observing the direction of motion of particles in the water on the UTV screen.

A Hydro Products model 403A temperature probe, with a measurement range of 0° to 40° C and an accuracy of ±0.5° C was used with a model 401 readout module located in the monitor room. Identical temperature readings were obtained with those readings taken with a mercury bulb thermometer at the UTV site.

Behavioral observations were made during both dawn and dusk periods. The videcon camera used in the UTV made nocturnal observations impossible without the use of artificial lights.

Late in the study an effort was made to determine vertical and horizontal distances travelled by the fish from the burrow by placing a grid of small markers made from 2.5 inch long carriage bolts at specified distances from the burrow.

Vertical heights above the burrow were measured by markers placed certain distances behind the burrow opening on the line of sight of the camera to the burrow (Text-fig. 1). By knowing the distance from the camera to the burrow, from the burrow to the marker, and the viewing height of the camera off the bottom, the vertical height of the fish could be determined by the use of similar triangles as long as the fish was directly above the burrow. The position of the fish in relation to the burrow was fairly easily determined by the relative size of the fish on the screen and by its image sharpness in the limited depth of field of the camera.

Definition of Behavioral Actions

It is necessary to define the actions of the animal which are to be measured. Ideally, the entire behavioral repertoire of an organism should be described. For the purposes of this paper only those actions concerned in the daily activity measurements will be defined. No organizational grouping of behavioral actions used in past studies has apparently fulfilled the needs of subsequent workers since it seems each study has required the modification of a previous or formation of a new hierarchy of actions. This was also true with the behavior of *Opistognathus aurifrons*. The behavior of the animal was di-
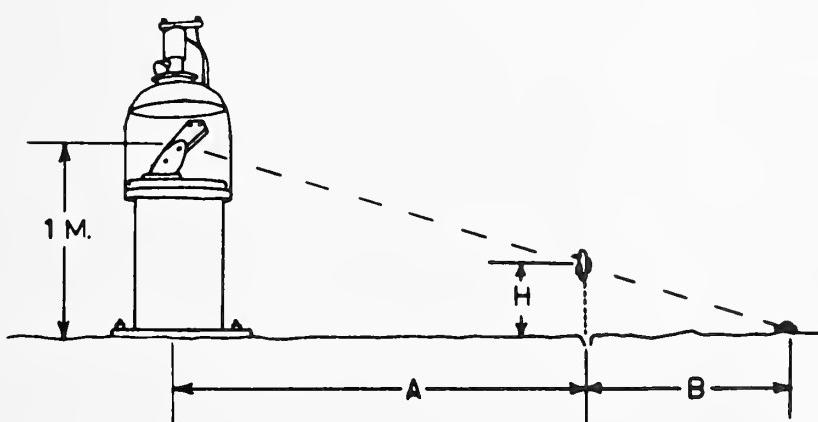
Text-fig. 1. The method used for the determination of the height (H) of a jawfish above its burrow at the UTV site. When the fish is in line with the distance marker (C), its height above its burrow in meters equals $\dfrac{\text{B in meters}}{\text{A + B in meters}}$.

vided into six general groups: 1) locomotory movements, 2) burrow oriented, 3) feeding oriented, 4) maintenance activity, 5) interspecific oriented, and 6) intraspecific oriented.

Locomotory Movements

Hover. The fish maintains itself in position above the substrate in an anterior-posterior vertical orientation by an alternate beating of the pectoral fins and a beating of the caudal fin, the posterior one-half of the body, the posterior part of the dorsal fin, and the anal fin. This is shown in Text-fig. 2a and 2b in lateral and ventral views. The dorsal, anal, and caudal fins are moderately spread while the pelvic fins may either be held tightly against the body (Text-fig. 2c) or held out at a right angle from the body (Text-fig. 2d).

The movements of the pectoral and caudal fins are co-ordinated so that the thrust produced by the motion of the caudal fin and the posterior portion of the body counteracts the tendency by the stroke of one of the pectoral fins to move the upper torso laterally. In this manner the cephalic portion of the body is maintained in a stable position.

The pelvic fins are held out from the body most often during periods of very low current speed. The extension of the pelvic fins may well aid in balancing the animal. This is the typical fin position assumed by fish in aquaria since currents there are practically non-existent. At current speeds greater than 0.03 knot the pelvic fins are usually held posteriorly against the body.

The density of seven live Florida specimens was determined utilizing a beam balance for weight determination and a graduate cylinder for volume measurement. A mean value of 1.04 g per cm^3 was obtained which makes *O. aurifrons* slightly denser than sea water (1.02 to 1.03 g per cm^3). Fish in aquaria under conditions of zero current speed were observed to stroke each pectoral fin at a rate of 80 to 95 strokes per minute in order to maintain a stationary position in the water column. The animal produces forward (upward) thrust with movements of the pectoral fin as shown in Text-fig. 3. The dorsal and ventral rays of the pectoral fin are brought forward while the medial rays of the fin lag (Text-fig. 3a) on the upstroke. On the downstroke all of the rays are brought back together (Text-fig. 3b).

To maintain its hovering position above the burrow during periods of high current speed, the fish must swim at an angle to the vertical with its head oriented into the current. The angle of the anterior-posterior axis of the body with the vertical increases with the intensity of the current. At zero current speed this angle is zero degrees, at 0.15 knot the angle is 45 degrees, at 0.20 knot the angle is 60 degrees, and at 0.25 knot the angle is 75 degrees. Currents over 0.30 knot in speed require *O. aurifrons* to swim practically horizontally in the water column. The beating rate of the pectoral and caudal fins is consequently increased during periods of increased current speed.

Movement Forward and Rearward. Forward movement by *O. aurifrons* is accomplished by simply increasing the beating rate of the caudal and pectoral fins. Rearward movements, however, can be carried out in several ways. The fish can move downward (usually rearward) by decreasing the fin beating rate. The fish can also move rearward due to its density being greater than seawater and reducing resistance to rearward movement by folding the pectoral fins forward. Finally it is possible for the animal to propel itself rearward by beating the pectoral fins in a reverse manner from that used in frontward movement.

Turn (Maneuvering) in the Hover (roll, pitch, yaw). Lateral turning (yaw) from the hover is accomplished by lessening the thrust or missing completely one or more strokes by one pectoral fin and bending the body laterally (Text-fig. 4). The thrust differential produced causes the body to turn laterally toward the side of lessened thrust.

Roll and pitch are accomplished in different manners. On the alternating downstrokes of the pectoral fins, either the dorsal or ventral rays can be brought back first. If this is done, the thrust produced is not on a line with the body axis. If the ventral portions of both pectoral fins are brought back first, a pitch in the ventral direction is accomplished. If the dorsal portions are brought back first, the pitch is in the dorsal direction. If the dorsal portion of one pectoral fin and the ventral portion of the other are brought back first, a roll is produced. What is henceforth referred to as a "turn" is a turning of the animal in the water column which can involve any or all of the roll, pitch, and yaw movements described.

Burrow Oriented Actions

Burrow oriented actions fall into two general categories: 1) those concerned with the burrow as a refuge from predators and a nocturnal resting place, and 2) those actions concerned with maintenance of the burrow.

Tailfirst Entry. The fish enters the burrow caudal-end first while backing slowly. As outlined previously, the fish may passively retreat due to gravity and currents or actively swim rearwards. The pelvic fins are folded against the body during this and all other entries in order to clear the burrow opening.
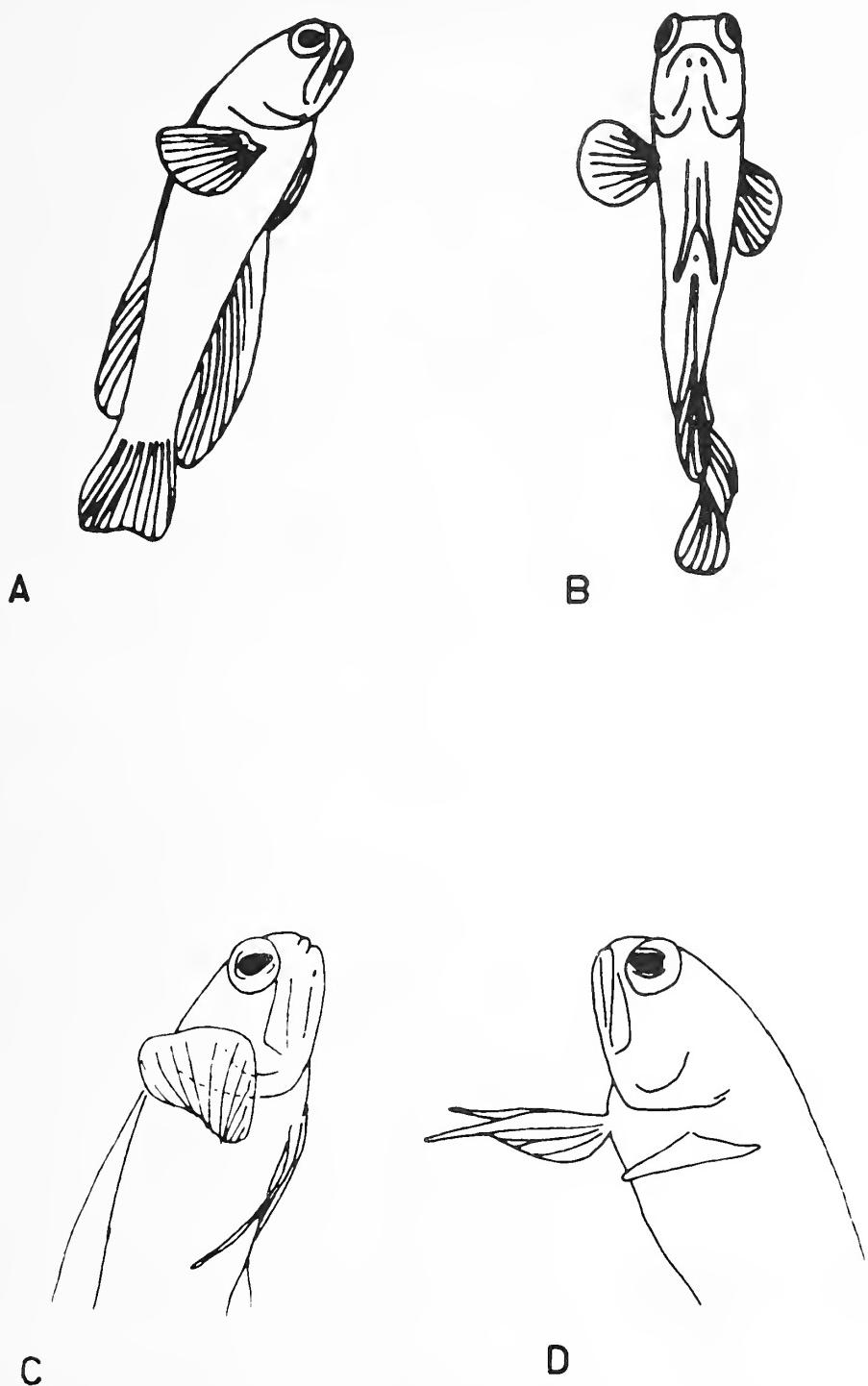
TEXT-FIG. 2. The action of "hover": A) lateral view; B) ventral view; C) "hover" with the pelvic fins held against the body; D) "hover" with the pelvic fins extended.
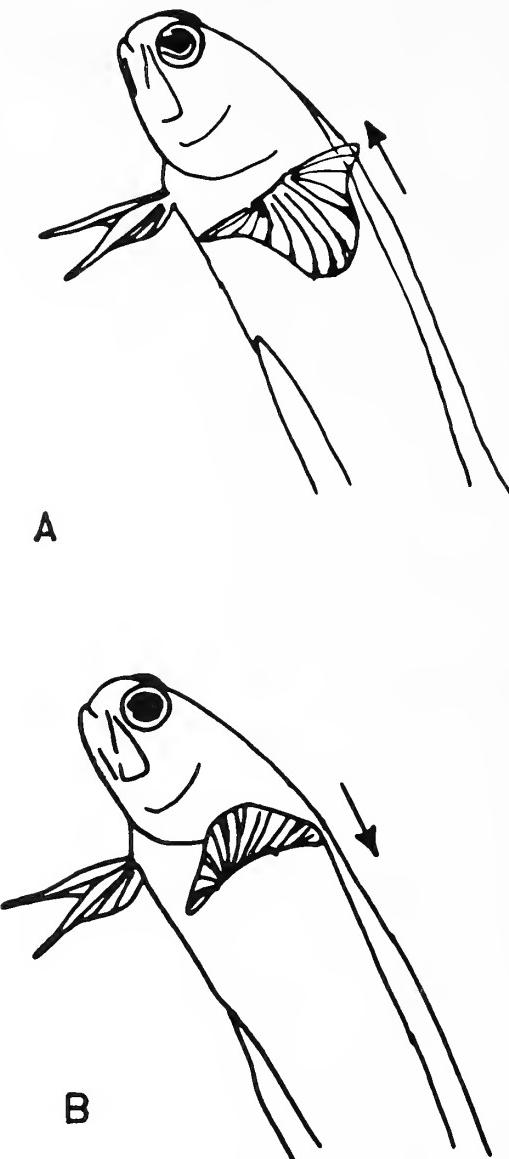
TEXT-FIG. 3. Movements of the pectoral fins during hovering. A) The upstroke of the pectoral fin with the medial rays lagging. B) The downstroke of the pectoral fin with all rays brought back together.
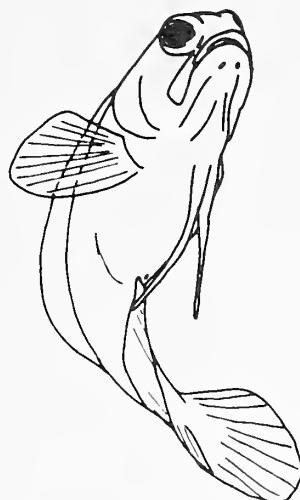
TEXT-FIG. 4. The lateral turn (yaw).

Headfirst Entry. The animal turns from a normal hovering position in the water column and swims rapidly head foremost into the burrow.

Tailfirst to Headfirst Entry. The fish backs toward the burrow tailend first until the caudal fin is immediately above the opening. The jawfish then turns 180° and enters the burrow head foremost.

Exit. The fish emerges head first, using the pectoral fins for propulsion. The pelvic fins are held posteriorly against the body until the animal is clear of the mouth of the burrow. The pelvic fins may then be extended.

Sit. The animal maintains position in the burrow opening with only the head (to the level of the opercular margin) exposed. Methods used for holding this position include sitting on the sides of sloping burrow entrances and wedging the body in the opening.

Cover Burrow. The jawsh enters the burrow tailfirst and as it descends will bend laterally or ventrally and pick up a small stone or shell in its jaws. This stone is released covering the opening as the head of the fish passes into the burrow. This action is usually performed at dusk or when predators approach.

Adjust Cover. The stone or rock covering the burrow may be adjusted in its position by the fish pushing up from inside the burrow and moving the stone with its head.

Uncover Burrow. The burrow is uncovered by the fish by moving forward in the burrow and pushing the covering stone out of the way with its head. The stone may then be moved by picking it up in the jaws if it is still blocking the opening. The action is usually performed at dawn or after the passing of predators.

Resting. That position within the burrow, whereby the fish rests on the substrate. All fins are folded, with the possible exception of the pectoral fins. This position is noted only at night. There is no apparent opening or closing of the mouth or the opercular apparatus.

Actions Related to the Maintenance of the Burrow

Dig. This action can be divided into three sub-actions which are not discreet motor patterns, but appear to have different functions.

A. Within burrow: Sand is scooped up in the mouth inside the burrow and it is then deposited outside the burrow near its margin (Plate III, fig. 4). When carrying sand, the branchial apparatus of the fish is expanded, the mouth closed, and the gill covers slightly open (Plate III, fig. 5). The dark line bordering the isthmus which is normally hidden is visible when the mouth is full of sand.

During periods of high current speed, the jawfish will use the current to its advantage in digging from the burrow. Rather than expelling the sand on or beyond the margin of the burrow, the fish will expel the sand vertically from its mouth without emerging fully from the burrow opening. The current will carry the sand over the burrow margin before it can fall.

B. Retrieve sand: Sand is scooped into the mouth at some distance from the burrow (Plate IV, fig. 6) and brought directly to the margin of the burrow where it is expelled. Again the dark line bordering the isthmus is visible when sand is being carried in the mouth. Movement of the caudal and pectoral fins is extremely rapid when swimming with the sand in order for the animal to stay above the bottom with this added weight.

C. Remove sand: Sand is scooped up from the margin of the burrow and carried some distance from the burrow where it is expelled. Again, the isthmus line is visible and the swimming rate rapid.

Retrieve Rock. This action, like "dig," is divisible into three sub-units.

A. Recover rock: A small stone or shell is picked up in the jaws and brought to the burrow where it is deposited on the margin. Carrying rocks differs from carrying sand in that the rock is held in the jaws while sand is carried inside the mouth. Also, sand must be forcefully expelled while the rock can be released by simply opening the jaws.

B. Remove rock: A small stone or rock is picked up in the jaws from the burrow margin and carried some distance away where it is deposited.

C. Remove rock from within burrow: The fish picks up a rock in its jaws inside the burrow, emerges headfirst, and drops the rock on the burrow margin (Plate III, fig. 7).

Adjust Rock. This action is performed with the body in the burrow with only the head exposed. Rocks on, or near, the burrow margin are picked up in the jaws and positioned on the margin. Often upon placing the rock in position, the jaws of the fish are not released and the rock is moved with the head to produce a more suitable resting place for it. Rocks may also not be removed from their original position but simply moved slightly to improve their positioning and that of surrounding rocks.

Actions Associated with Feeding

Thrust. The fish moves rapidly forward from a hovering position through the use of the pectoral and caudal fins. The animal comes to a quick stop through the use of the pectoral fins (Text-fig. 5).

Snap. The jawfish ingests a food particle by opening the mouth and creating a slight inrush of water by flaring out the opercular covers and spreading the branchiostegals (Text-fig. 6a and 6b). The line hidden under the maxillary is exposed when the mouth is opened, and the long axis of the pupil is oriented so the fish may see the food particle with binocular vision.
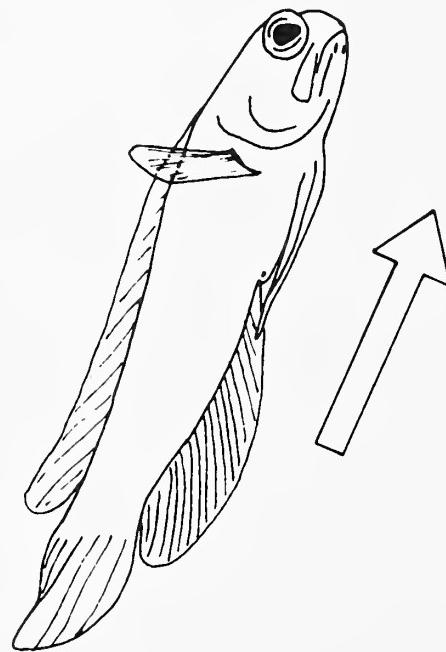
Text-fig. 5. The action of "thrust."

Reject. The food particle is ingested as in a snap, but is quickly expelled from the mouth by a pulling in of the gill covers and branchial apparatus.

Maintenance Activity

Although the jawfish possesses several apparent maintenance activities, these will not be described since they were not quantified diurnally.

Actions Concerned with Interspecific Relationships

The interspecific relationships of *O. aurifrons* have previously been dealt with by Colin (1971). The action, chase, was the only activity for which diurnal data are available.

Chase. The act of chasing an intruding fish with jaws spread. It usually occurs within 20 cm of the burrow and the distance a fish is pursued varies a great deal. Swimming is carried out rapidly with the pectoral and caudal fins.

Actions Concerned with Intraspecific Relationships

A variety of intraspecific relationships exist among individuals of *O. aurifrons*. Some of these have already been mentioned by Colin (1971), while others are described below:

Arch. This is the "lateral display" of Leong (1967). I feel the term "lateral display" is more suited for a different action involved in territorial defense where one fish turns laterally toward another, with the body oriented nearly vertically and spreads the mouth, branchial apparatus, and isthmus to their maximum while the head is shaken laterally. Leong (1967) did not describe such an action for *O. aurifrons*.

The "arch" is performed by the male fish. This fish, swimming in the water column, will orient its body laterally toward a female and assume a horizontal position. The caudal and cephalic ends of the body are bent upward and the dorsal, anal, and caudal fins are spread to their maximum. Immediately after the body is bent, the branchial apparatus and the isthmus are dropped and the mouth is opened (Plate V, fig. 8). The spread of the mouth is not as great, however, as it is during the aggressive "lateral display." The isthmus, maxillary, and branchiostegal lines are all clearly displayed, and the arch position may be held for several seconds. Often after this action, both fish will enter one burrow for a period of several seconds. It is for this reason that the "arch" is considered as courtship behavior rather than some other type of intraspecific behavior.

Brood. The male fish broods the eggs orally (Text-fig. 7) and is positioned usually directly above the burrow entrance in a hovering attitude. The maxillary line is clearly exposed and the branchial apparatus not greatly expanded.
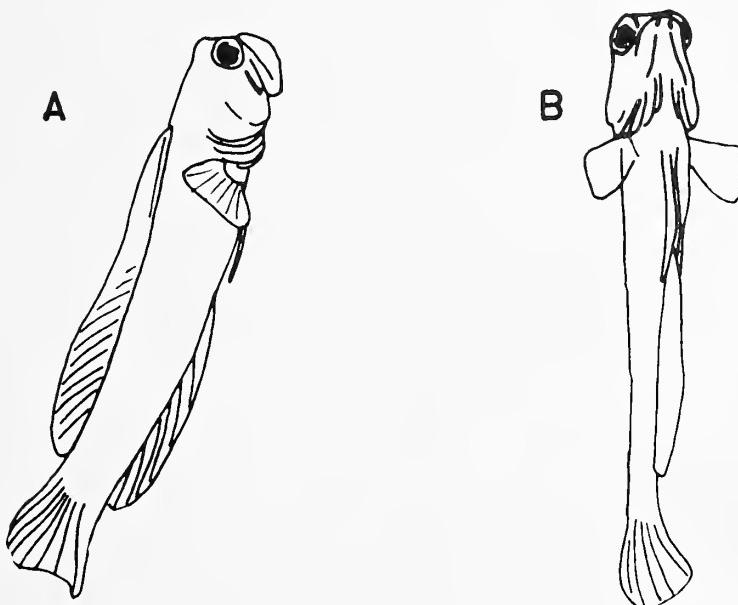
TEXT-FIG. 6. The action of "snap"; A) dorso-lateral view, B) ventral view.
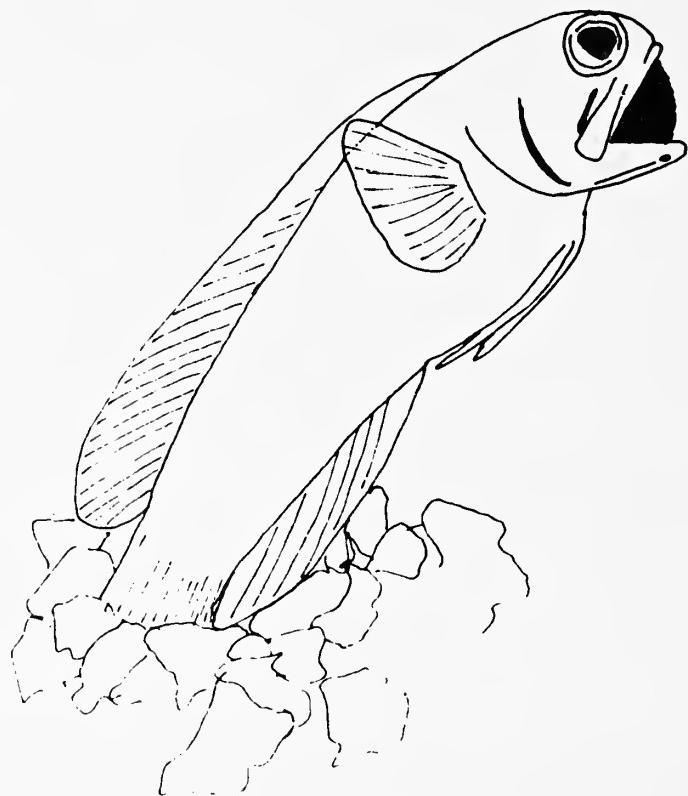
TEXT-FIG. 7. The action of "brood."

The mouth is usually open but it can be nearly closed while carrying the eggs. Closing the mouth when the egg mass is being carried causes a consequent expansion of the branchial apparatus. The head is expanded somewhat laterally and the isthmus lines are apparent in a front view of the animal.

DAILY ACTIVITY PATTERNS AND BEHAVIORAL CORRELATIONS

In situ studies of coral reef fishes have been carried out only recently, and work dealing with *in situ* measurements of daily activity of coral fishes is extremely scarce. Some of the noteworthy studies are the following. Youngbluth (1968) worked with the Hawaiian, parasite-picking wrasse, *Labroides pthirophagus,* and determined feeding (cleaning) rates of these fish at two hour intervals during the day. No significant difference was found between morning and afternoon rates but feeding rates varied on different reefs. Albrecht (1969) studied the fanning of the nest by the pomacentrid, *Abu-*

defduf saxatilis, in relation to depth and time. His observations were both diurnal and nocturnal. Myrberg (in press) worked with the daily patterning of various sonic patterns in the pomacentrid, *Pomacentrus partitus,* and included observations both in the field and in laboratory aquaria.

During the present study, two individuals of *O. aurifrons* were sufficiently close to the UTV camera so that detailed observations and rather precise measurements of behavior could be carried out (2m). Four other individuals were approximately 8m away and although their position in the water column could be observed, lack of detail precluded behavioral measurements. The jawfish, male and female, had their burrows 60 cm apart.

This pair was closely observed for 25 days during the summer of 1969 and the frequency of certain actions was recorded for 30 minutes during each of seven periods throughout the day. Fifteen minutes of each period was de-

voted to the activities of each of the subjects. The onset of the periods was altered so that they maintained the same relative position in regards to the changing length of the daylight period. For example, the periods originally at 9:00 AM on June 24 was moved to 9:18 AM on Sept. 7 to keep the same position in total day length. The days spent observing and recording the behavior of *O. aurifrons* on the UTV included June 24 to 30, July 17 to 24, August 2 to 6, August 26 to Sept. 3, and Sept. 5 to 8, 1969. Preliminary observations but no quantification of behavior were carried out on June 4 to 6.

For purposes of recording various activities on a 12-channel event recorder, activity was broken down into four major groups: 1) feeding, 2) burrow oriented, 3) interspecific, and 4) intraspecific. Whenever possible an "indicator action" was selected to reflect the level of a certain type of activity. This "indicator action" is often not the most direct measure of a major activity group. Sevenster (1961: 17-18) for example, correlated the number of "zigzags" of the males of *Gasterosteus aculeatus* with the frequency of the male leading the female, a purely sexual activity. He then used the more easily observed "zigzag" as a measure of the sexual activity of the male instead of the less easily observed action of leading.

For *O. aurifrons* the "snap" was selected as an indicator action for feeding activity since it was easily observable and reflected reasonably well the actual food intake of the animal. The actions of "thrust" and "turn" were also considered possible feeding actions and their frequencies, along with those of "snap," were measured during the period June 24 to 30. Regression lines were calculated from these data (Text-figs. 8 and 9) and clear correlations were noted among the occurrences of these actions. Therefore, each could be considered as elements of feeding behavior and it was necessary to determine only the frequency of "snaps" in succeeding behavioral measurements.

Measurements were also made of the amount of time spent by a given fish in the water column above its burrow. The "Percent time in the water column" was subsequently determined, with those activities directed away from the water column, such as retrieval of sand, not being included in this percentage.

The seven observation periods during the day were numbered chronologically for reasons of analysis. Text-fig. 10 illustrates feeding activity, as reflected by the mean number of "snaps" per 15 minute period for each of the seven periods of the day. The feeding rate ("snaps") is fairly constant over the entire day considering all environmental conditions. Reaction to specific environmental factors such as current speed and light intensity will be examined later.

The relationships that exist among the actions involved in burrow oriented activity are more complex than those involved in feeding. Digging from within the burrow is commonly seen, but it cannot be considered to reflect the nature of the fluctuations shown by related activities such as: remove sand, retrieve rock, remove rock, and adjust rock (Text-fig. 11 and Table 1). These, therefore, must each be considered separately. Text-fig. 11, illustrating mean digs per 15 minutes, shows a strong peak (10.4) at the fifth period (3PM—start of study) subsequent to a moderate, but fairly consistent digging rate during the first four periods (4.6 to 7.3). There is a sizable decrease in the rate after the fifth period with a rate of only 1.6 at the seventh period (7PM—start of study).

Table 1 presents the mean values per 15 minutes of three other burrow oriented activities: removal of sand, retrieval of sand, and adjustment of rocks. The latter two show marked increases in the final three periods of the day with their greatest values being in the last period. Removal of sand differs since its peak value is during the first period of the day with only a slight increase in the afternoon.

The frequency of the activity, chase, is shown in Text-fig. 12. The increased frequency of this activity in the last three periods of the day was probably due to an increase in the swarming behavior of various labrids at that time. *Halichoeres bivittatus* and *H. garnoti* were very active during the final period of the day, often causing a jawfish to prematurely close its burrow for the night.

The frequency of flight reactions of *O. aurifrons* from other species of fishes was difficult to determine objectively. Often an approaching fish could not be seen on the UTV but the jawfish would flee to the burrow. Conversely, the fish has been observed to move rapidly to the burrow during the approach of a large fish, then emerge several seconds later with a mouth full of sand, as noted during a "dig." Such occurrences in the face of two equally possible stimulus situations preclude the use of possible flight responses in measurements of daily activity. Other aspects of interspecific activity have been considered elsewhere (Colin, 1971).

The occurrence of the probable male courtship pattern "arch," is shown in Text-fig. 13. Percent of total "arches" is plotted rather than a mean value since the sample size was rather small (35). Arching was most prevalent early in the morning and during late afternoon periods. Aquarium observations supported this finding, most arches occurring shortly after the burrow had been uncovered in the morning.

TEXT-FIG. 8. Correlation of the frequency of the actions of "snap" and "thrust."
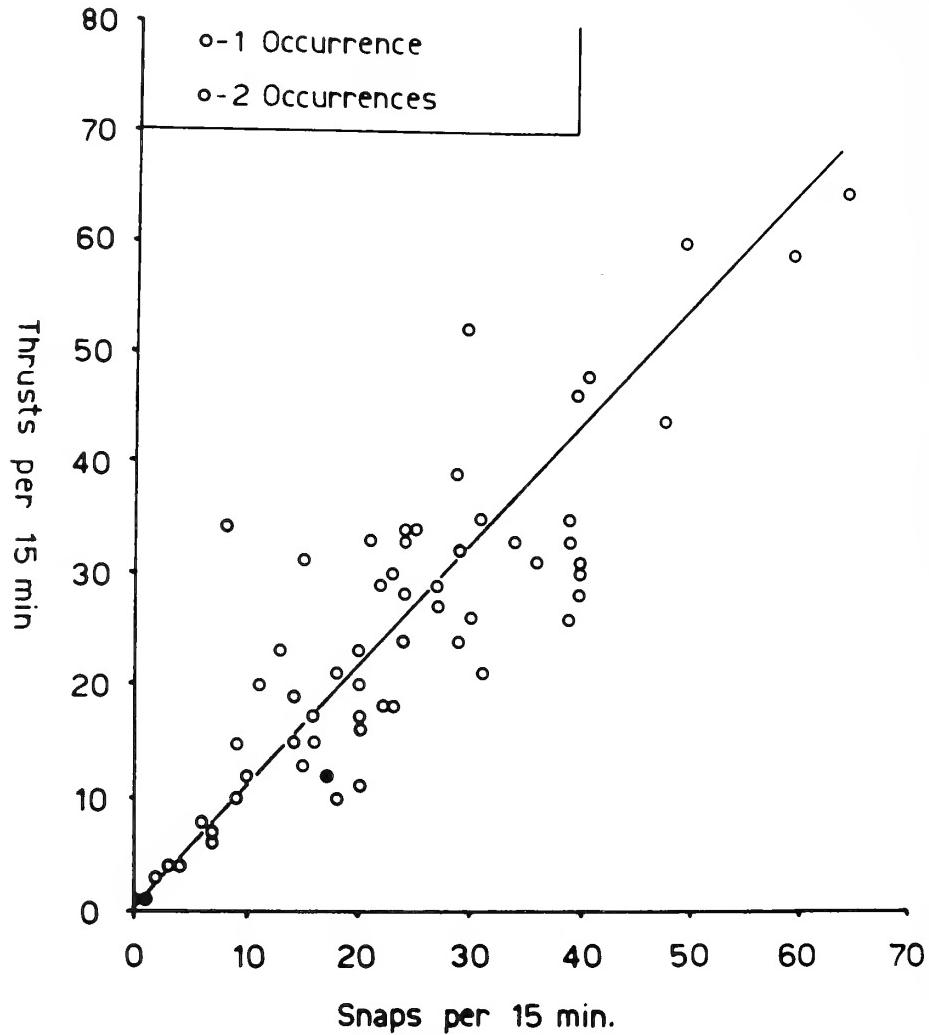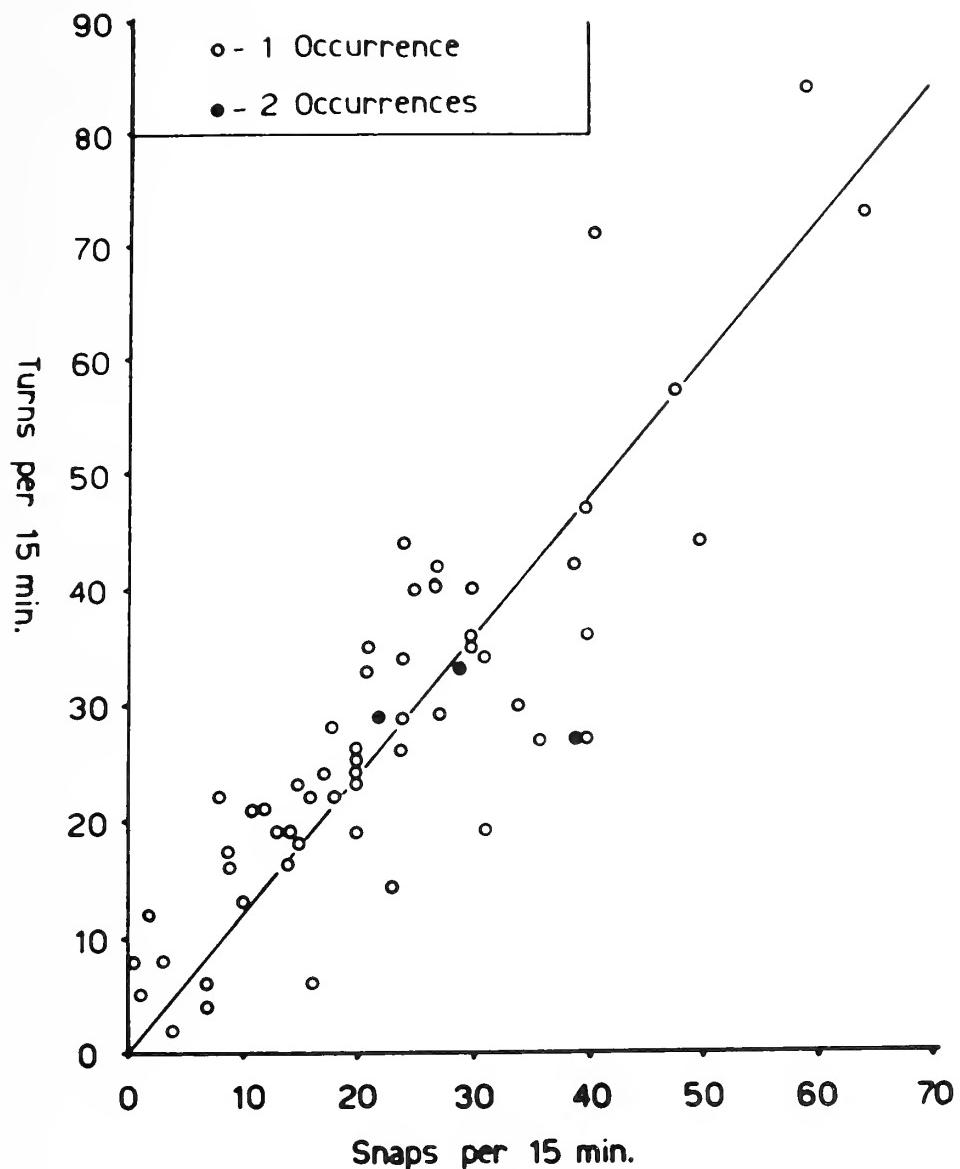
TEXT-FIG. 9. Correlation of the frequency of the actions of "snap" and "turn."
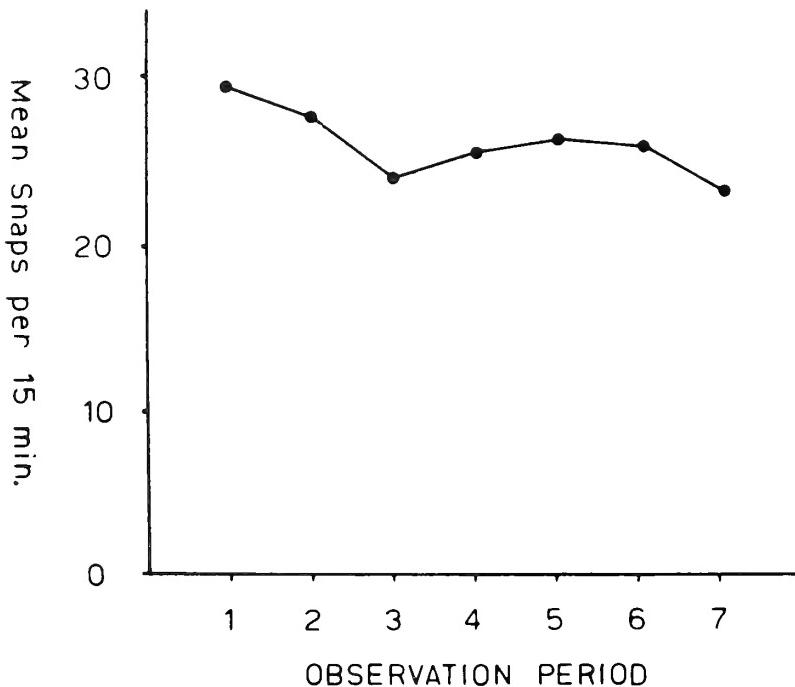
TEXT-FIG. 10. Diurnal patterning of the mean "snap" frequency of two specimens of *Opistognathus auROWSfrons* for a period of 25 days at the UTV site, Bimini, Bahamas.

TEXT-FIG. 11. Diurnal patterning of the mean "dig" frequency for two specimens of *Opistognathus aurifrons* for a period of 25 days at the UTV site in Bimini, Bahamas.

TABLE 1. MEAN FREQUENCY OF BURROW ORIENTED
ACTIONS PER 15 MINUTES FOR A
PERIOD OF 25 DAYS

Period	Retrieve Sand	Remove Sand	Adjust
1	0.21	1.68	0.31
2	0.02	0.08	0.18
3	0.59	0.02	0.25
4	0.26	0.00	0.13
5	1.10	0.51	1.48
6	3.83	0.25	1.05
7	4.24	0.05	1.81
Total Number of Periods Observed 231			
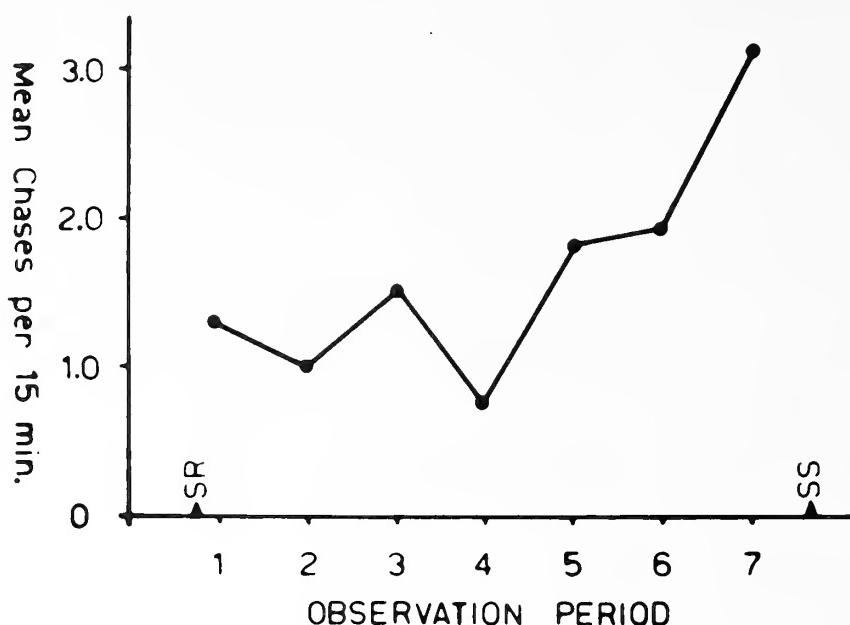
Text-fig. 12. Diurnal patterning of the mean frequency of "chase" for two specimens of *Opistognathus auritrons* for a period of 25 days at the UTV site in Bimini, Bahamas.

Text-fig. 13. Diurnal patterning of "arch" (given as percent of occurrence) for a period of 25 days at the UTV site, Bimini, Bahamas.

RELATIONSHIP BETWEEN FEEDING AND BURROW ORIENTED ACTIVITIES

The two major time-consuming activities of the field subjects were feeding and burrow oriented behavior. These two activities were inversely related since the first involved being in the water column and the second did not. Text-fig. 14 shows the mean number of "snaps" and "digs" against the percent time in the water column. As the time in the water column increases, the burrow oriented action ("dig") decreased and the water column oriented action ("snap") increased. However, the number of "snaps" or "digs" was not directly proportional to the percent time in the water column. The "snap" value at 50 percent time in the water column was only one-quarter of the value at 100 percent time in the water column, not one-half as would be expected if the feeding rate was constant over the entire period spent in the water column. Percentage values below 33 percent time in the water column were not observed in the field except when the fish remained in its burrow for known or unknown reasons for the entire 15 minutes. This is also discounting a few "aborted" periods where the jawfish was frightened into its burrow after a few minutes of normal behavior and remained there for the remainder of the observation period.

The explanation for the seeming paradox rests with both the behavior of the fish while digging and the length of the observation period (15 min.). Brief periods of hovering always interrupted separate bouts of digging, and such periods accounted for at least 33 percent of a given observation period.

There is little diurnal variation in the mean percent time spent in the water volumn as is shown in Table 2. Mean percentages from a low

TABLE 2. DIURNAL VARIATION IN MEAN PERCENT TIME IN THE WATER COLUMN

Observation Period	Mean Percent Time in the Water Column	Number of Periods
1	91.0	25
2	87.2	28
3	90.8	25
4	85.6	27
5	84.7	18
6	83.8	24
7	90.7	16
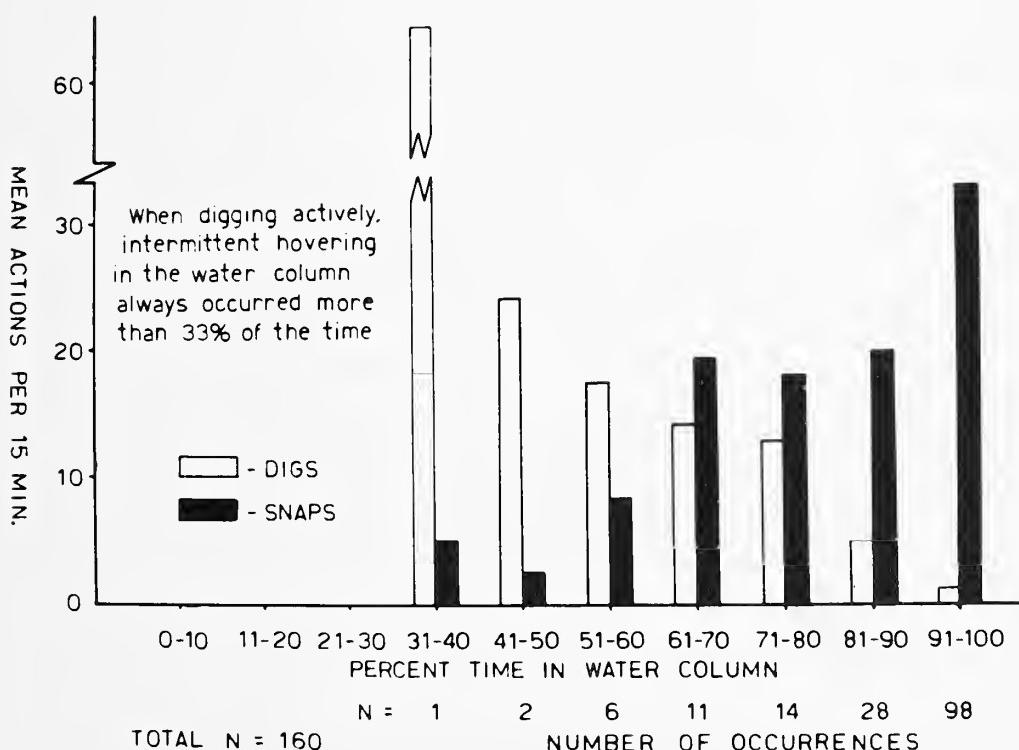
TEXT-FIG. 14. Relationship of mean "dig" and mean "snap" frequency to percent time in the water column.

of 83.8 to a high of 91.0 indicate that water column oriented behavior (feeding) dominated the total daily activity. This does not, however, reflect feeding effectiveness ("snap" rate) which can be altered by environmental conditions.

Effect of Current Speed Upon Various Activities

Current speed affects the "snap" frequency in combination with certain other conditions. Text-fig. 15 shows the mean "snap" frequency during observation periods when the current speed was greater than 0.20 knot. There is a sizable decrease in the mean "snap" frequency during the low light periods (periods 1, 2, and 7) in which high current speed was encountered.

The "snap" frequency for periods where the current was 0.20 knot or less is shown in Text-fig. 16. In this case the "snap" rate was nearly constant throughout the day with only a slight dip at the third period.

The values at low light periods during high current speed shown in Text-fig. 15 are therefore being masked in Text-fig. 10 by the greater occurrence (85 percent of all observations) of currents 0.20 knot or less. During the winter, however, shorter days and generally less consistent water conditions will no doubt increase periods of low ambient light and of high current speed.

It seems then that neither high current speed (0.20 knot or greater) nor low light conditions alone could produce any significant decrease in the frequency of "snaps." In fact, the mean "snap" frequency at periods 3 and 4 is greater for currents above 0.20 knot (Text-fig. 15) than for currents of 0.20 knot or below (Text-fig. 16). During high current, low light conditions, the fish does not move far from the burrow and instead hovers with the anterior posterior axis of the body near horizontal directly above the burrow opening. I feel that *O. aurifrons,* being mainly a visual feeder, probably requires a certain level of both light and current speed to feed effectively. Variation in only one parameter does not apparently affect the feeding rate.

Digging is also somewhat related to the current speed as shown in Text-fig. 17. The mean values of "digs" in periods in which digging occurred was similar for currents of 0 to 0.10 knot and for currents greater than 0.10 to 0.20 knot. The mean "dig" frequency showed a significant decline (at least 95 percent separation for currents greater than 0.10 to 0.20 knot and greater
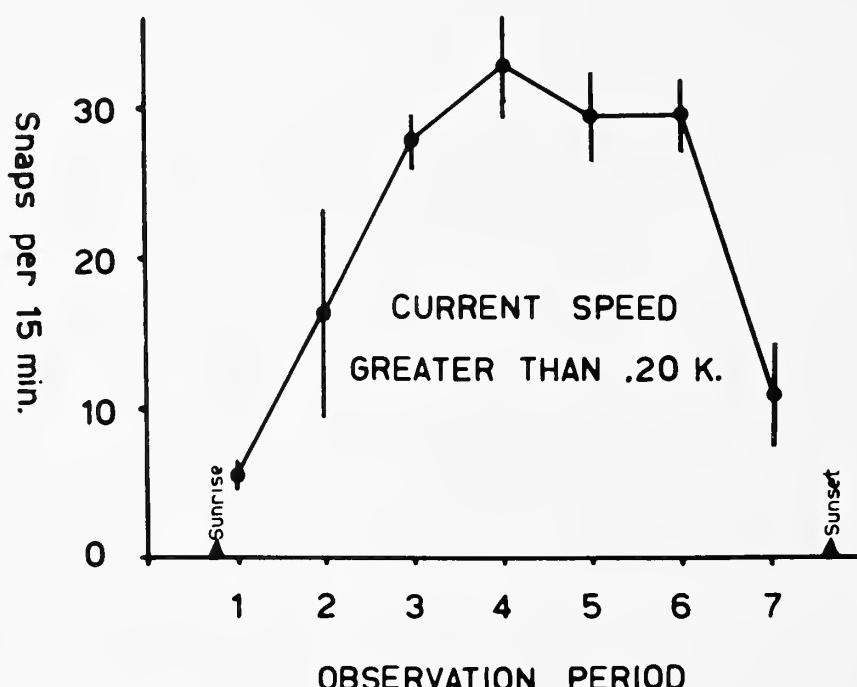
Text-fig. 15. Diurnal patterning of mean "snap" frequency of *Opistognathus aurifrons* when current speed was greater than 0.20 knot at the UTV site in Bimini, Bahamas.

than 0.20 knot), however, for currents greater than 0.20 knot. The reasons for this decline are still unclear.

The action of arching also seems to be related to the current. Text-fig. 18 plots the percentage of "arches" observed against various current conditions. Almost three-quarters of the "arches" occurred when current speed was 0 to 0.05 knot, while these currents occurred in only one-quarter of the observation periods. Current speeds of 0 to 0.10 knot were observed in 52 percent of the periods, yet over 90 percent of the arches observed occurred during this current speed regime. Additionally no arching was observed at current speeds over 0.20 knot. Since the arch is a complex posture supposedly directed at a female, the utility of performing this action under current conditions where the displaying fish is not quickly carried away by the current is obvious.

The percent time spent by the fish in the water column possibly varied with the current and this might easily explain the results shown in Text-fig. 15 (currents speed greater than 0.20 know versus "snap" frequency) and in Text-fig. 18 ("arches" versus current speed). But Table 3 shows that the mean percent time in the water column was independent of the current speed.

The parameter of current direction was recorded with current speed data but it showed no correlation with any behavioral measurements. The only change seen in the fish was a change in the direction in which they faced in order to swim into the current. During periods of slight or no current speed, the swimming direction of the fish seemed random except when an apparent food item was sighted.

EFFECT OF OTHER ENVIRONMENTAL FACTORS UPON VARIOUS ACTIVITIES

Temperature measurements were also made at the time behavioral data was taken, but stability of temperature during the study (29° to 31° C) precluded meaningful correlations with behavior. Temperatures near the winter low of approximately 20° C might produce considerably different results. Yellowhead jawfish, kept in aquaria, become very inactive at temperatures approaching 20° C and feed very sparingly. Below 20° C the animals spend most of their time in the burrow, and at 17° C appear near their lethal lower temperature limit.

Atmospheric conditions at the UTV site were also considered, qualitatively, as possibly influencing behavior. Numerous thunderstorms and
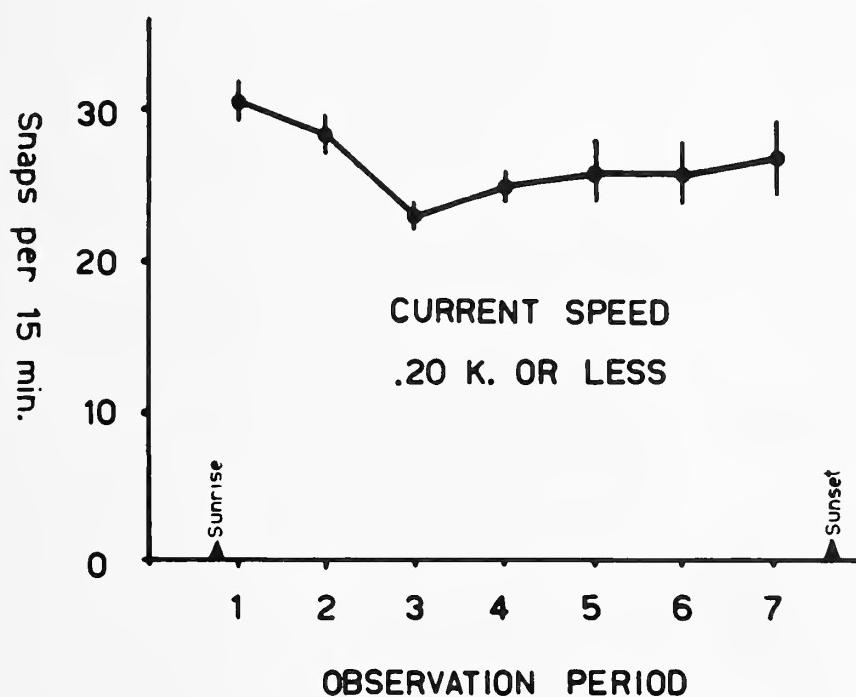
TEXT-FIG. 16. Diurnal patterning of mean "snap" frequency of *Opistognathus aurifrons* when current speed was 0.20 knot or less at the UTV site in Bimini, Bahamas.

heavy rain, which could be heard at the site via the submerged hydrophone, seemed to have no effect on the activity of the fish. Surging of currents on the bottom (depth 20 m) produced by surface waves resulted in movements of grass blades and bits of detritus. These occasionally rolled along the bottom and entered burrow openings. Such objects were quickly removed by the fish from the burrow.

Turbidity measurements were not made, but increased turbidity no doubt affects the activities of jawfish since any decrease of ambient light reduced visibility. Such reduction might well cut the feeding effectiveness of the animal as well as reducing its range of movement.

SONIC ACTIVITY

Extended listening has been unproductive in detecting any sonic activity by *O. aurifrons*. A hydrophone was positioned less than one-half meter from the fish at the UTV site for the duration of the study, and several hours were spent listening with hydrophones in laboratory aquaria at various times of the day, but the results from this monitoring have been negative.

TIME OF COVERING AND UNCOVERING THE BURROW AND NOCTURNAL BEHAVIOR

Opistognathus aurifrons covers its burrow opening in the evening with a small rock or shell and remains within the confines of the burrow until the morning when the rock is removed. Table 4 presents the results of numerous observations on the time and conditions of the opening and closing of the burrow.

The time at which the burrow is uncovered during the morning varies from ten to 12 minutes before to a very few minutes after sunrise. Mornings with clear skies tended to have early uncoverings and mornings with late uncoverings were usually overcast. The fish tended to rise two or three minutes after the first objects on the bottom were visible on the UTV. This time of first visibility, of course, varied with the sky conditions. Aquarium fish will uncover the burrow any time during the night if lights are turned on, but the time required for this to happen was generally longer (as much as 10 to 15 minutes) during late night and early morning hours. When the lights were turned on at the normal uncovering time, the fish usually removed the cover of the burrow in less than one minute.
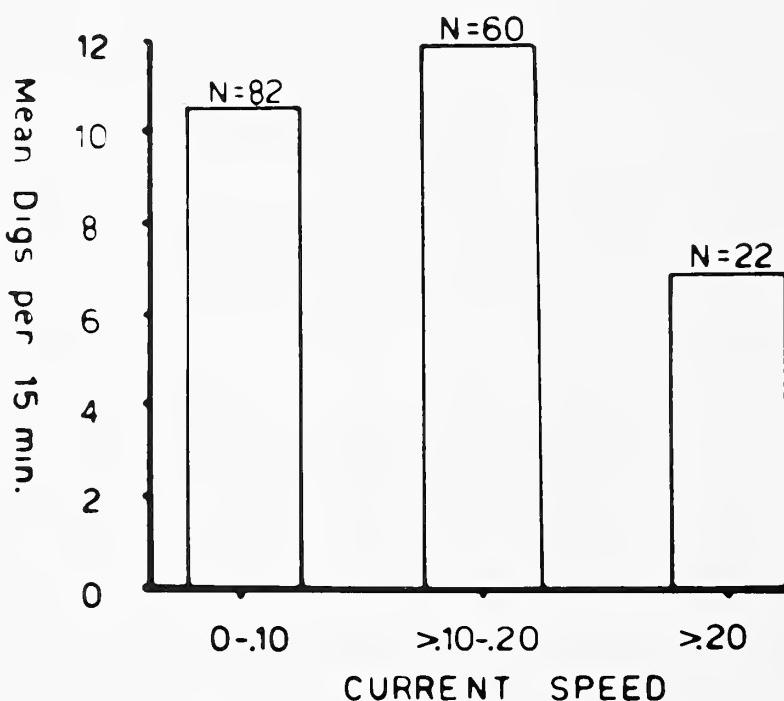
TEXT-FIG. 17. Relationship of current speed to the mean number of "digs" by *Opistognathus aurifrons* during periods in which digging occurred.
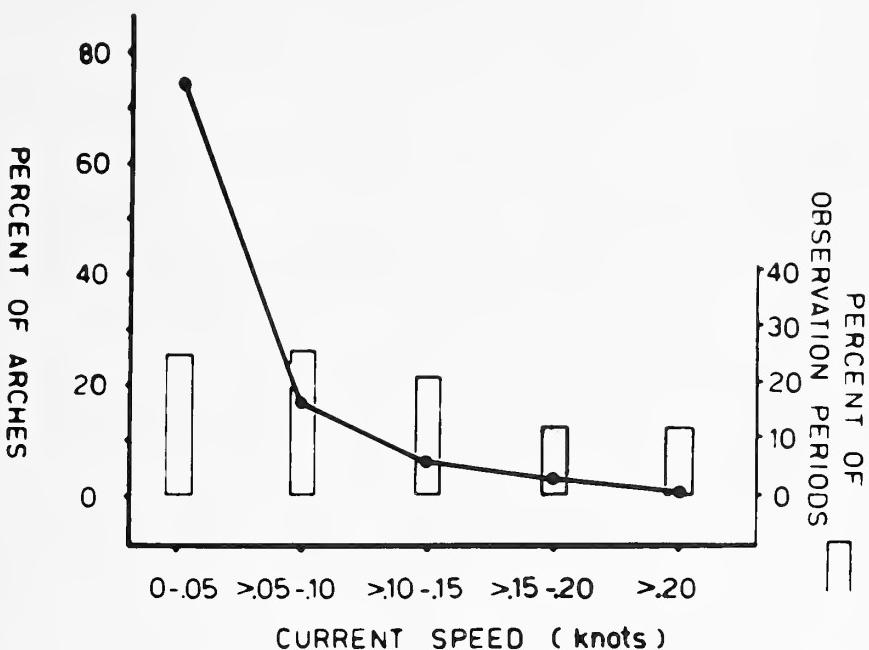
TEXT-FIG. 18. The relationship of percent of "arch" and percent of occurrence of various current speed regimes to the current speed.

TABLE 3. VARIATION IN MEAN PERCENT TIME IN THE WATER COLUMN WITH CURRENT SPEED

Current Speed	Mean Percent Time in the Water Column	Number of Periods
0.00-0.10 Knot	88.9	82
greater than 0.10-0.20 K.	85.9	60
greater than 0.20 K.	90.5	22

Light is probably a major controlling factor in determining the uncovering time. As day length changed, the time of uncovering the burrow was altered to match the changing sunrise time. On mornings when ambient light was low due to atmospheric conditions, this was reflected by a later rising time.

A different situation existed with respect to the time of covering the burrow in the evening. Times of from 92 minutes before sunset to six minutes after sunset have been recorded. However, many factors apparently entered into the determination of the closing time. The presence of other species in the area seems to have a definite effect. Large numbers of the fishes *Halichoeres bivittatus, H. garnoti,* and *Pseudupeneus maculatus,* browsing on the substrate near territories of *O. aurifrons,* often coincided with an immediate retreat to the burrow and a covering of the mouth of the burrow with a stone. If this occurred near dusk, the fish often remained in the hole for the night.

Light appeared to control the absolute limits of time for closing the burrow. One-third of all closings occurred between sunset and six minutes thereafter (maximum limit).

A series of night dives on colonies of *O. aurifrons* by the author on the Florida reef tract in 1969 showed no evidence of any nocturnal activity. This agrees with Starck's (1966) statement "at night it (*O. aurifrons*) has never been found, and is apparently inactive." Fish in various aquaria (40 to over 2000 liters) also remained in their burrows the entire night as evidenced by irregular frequent inspections.

TABLE 4. TIME OF UNCOVERING AND COVERING THE BURROW BY *Opistognathus aurifrons*

Uncovering in the morning Time in minutes before (+) or after (−) sunrise	Number of occurrences	Percent of occurrences
greater than +10	2	13.3%
+10 to 0	10	67.0%
−1 to −10	2	13.3%
greater than −10	1	6.7%

Covering in the evening Time in minutes before (+) or after (−) sunset	Number of occurrences	Percent of occurrences
greater than +20	9	33%
+20 to +10	1	4%
+10 to 0	8	29%
−1 to −10	9	33%

MORNING AND EVENING BEHAVIOR

The present section deals with those events which immediately followed opening and preceded closing the burrow. The activities occurring during these two periods of the day are extremely different.

Text-fig. 19 shows that after opening the burrow in the morning, the fish quickly entered the water column with a resultant decrease in the time spent in the burrow. The time spent within the burrow opening (neither completely out or in the burrow) reaches a peak five minutes after opening the burrow, but it never occupies a significant percentage of the fish's time. Feeding began as soon as the fish entered the water column; after only four minutes the frequency of "snaps" was practically equal to the mean daily frequency (see Text-fig. 10). Burrow oriented activities such as digging and retrieving were non-existent in the early morning period, and interspecific activity was rarely observed.

Actions which immediately preceded closing the burrow in the evening were considerably different than those following its initial opening. A striking increase in both "adjust rock" and "retrieve sand" (Text-fig. 20) demonstrated that individuals physically prepare their burrow for the night. The adjustment of rocks may serve to prepare the opening for its covering stone, and the retrieval of sand to hide the stones of the burrow rim or to provide for a better fit for the covering stone. One covering stone is not reserved for use day after day, but a suitable stone, often one-half of a bivalve mollusc shell, is selected shortly before dusk and placed near the burrow opening.

Feeding declined in the final minutes (Text-fig. 20), but a low level remained up to the moment that the burrow was closed for the night.

RANGE AND TERRITORY

The concepts of range and territory are separate entities. The word, range, implies the total area into which an individual confines its presence. Territory, however, is a somewhat more elusive concept bringing to mind that area which an animal "considers" its own and is willing to defend. Often the territory of an animal depends upon the type of intruder that is encountered. The burrow of *O. aurifrons* may logically be considered the "center" of both its range and territory with the level (or intensity) of defense decreasing rapidly with distance from that point.

The territory was defended only against those fishes that are nearly the same size or smaller than *O. aurifrons*. Its efforts at territorial defense are minor compared to other species of reef fish, such as members of the genus *Pomacentrus* (Emery, 1968a; Myrberg, in press). Small fishes would be chased from a circle 20 to 25 cm in radius, with the burrow at its center. Fishes beyond 'this distance were apparently "watched" but no aggressive actions were directed toward them.

The range of the yellowhead jawfish should be considered from two aspects. The first is the feeding range which in benthic feeding fishes includes horizontal movement. Since *O. aurifrons* is a plankton feeder, this range also included vertical movement above the bottom. Text-fig. 21 presents the modal height and also the greatest height seen in any one period during given periods throughout the day. Heights greater than one meter could not be quantitatively measured, since there was nothing visible behind the fish for sight reference. The greatest
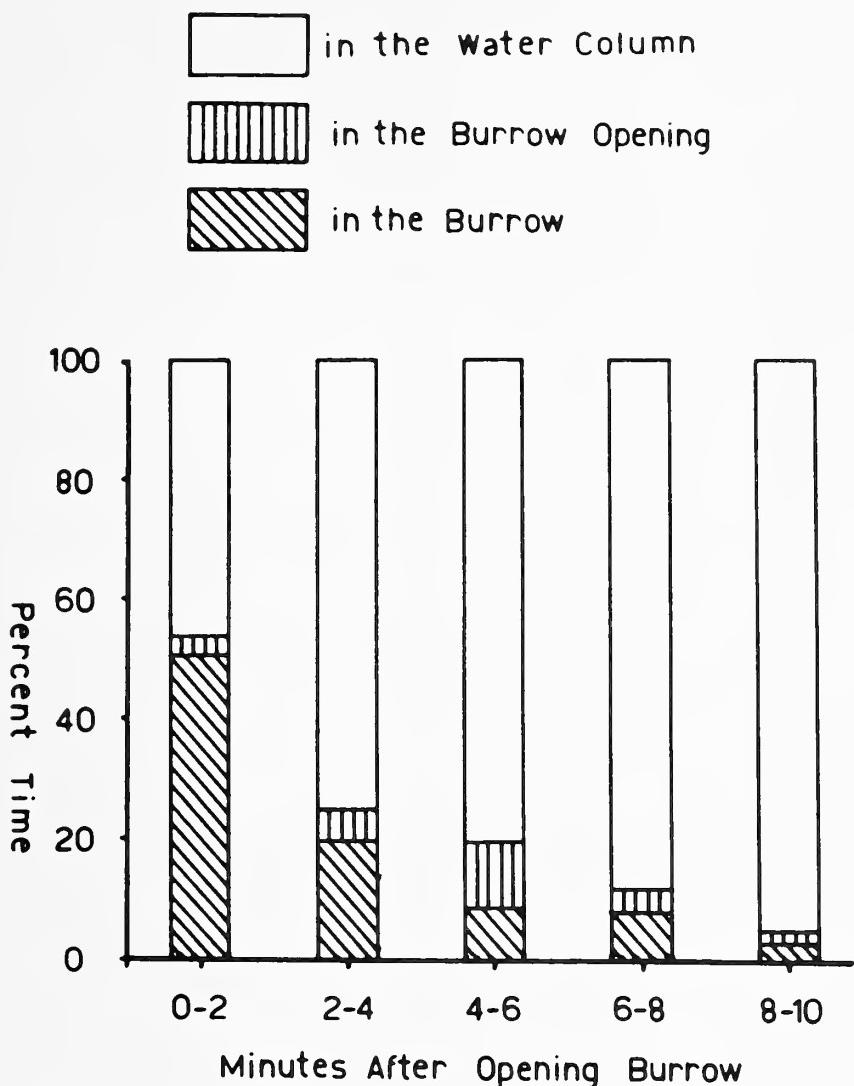
TEXT-FIG. 19. Percent of two-minute periods spent in various locations by *Opistognathus aurifrons* after opening the burrow in the morning at the UTV site in Bimini, Bahamas. All values are the mean of six observations.
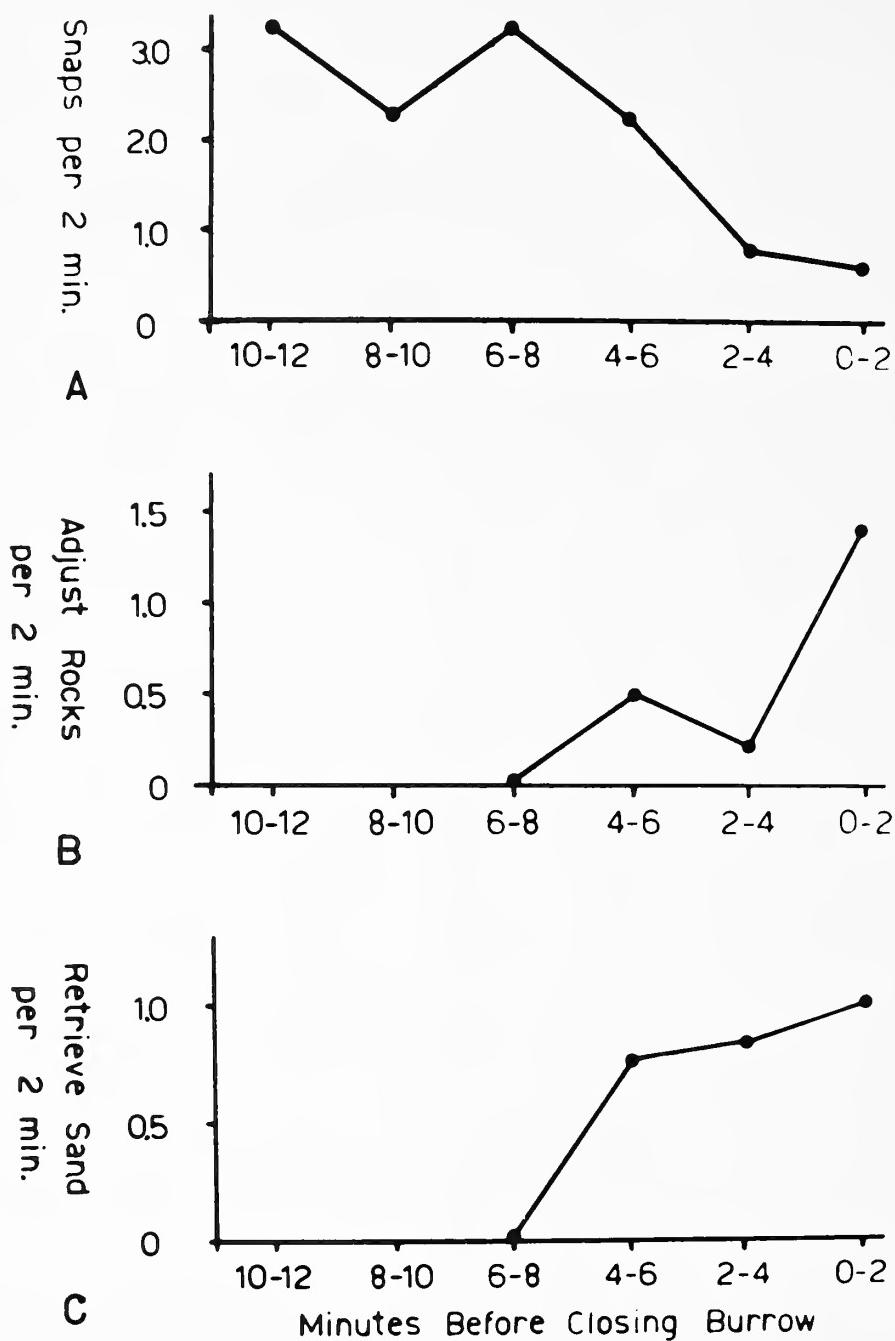
TEXT-FIG. 20. A) The occurrence of "snap" by *Opistognathus aurifrons* before closing the burrow for the night. Mean of ten observations. B) The occurrence of "adjust rock" by *O. aurifrons* before closing the burrow for the night. Mean of ten observations. C) The occurrence of "retrieve sand" by *O. aurifrons* before closing the burrow for the night. Mean of ten observations.

height ever reached by the animals was estimated about one-and-one-half meters above the bottom.

The low heights reached during the morning and evening hours were probably the result of low light levels, and this apparent adaptation no doubt provided greater chance for avoidance of predators. The horizontal component of the vertical ranging of the animals was at most 2 m, but seldom more than 1 m from the burrow opening.

The second aspect of range to be considered is that having to do with rock and sand retrieval. The number of rocks which a fish considers suitable for its burrow in a given area must, of course, be limited. If these are used in burrow construction, the fish must then extend this range to retrieve additional ones. At the UTV site the range for rock retrieval was approximately 2 m. The fish would take a zigzag course outward inspecting various stones until a suitable one was found. The return course was direct, straight to the burrow.

The range for retrieval of sand was considerably smaller (generally less than ½ m) due, no doubt, to the easy availability of suitable sand.

Rock stealing (i.e., taking rocks from the burrows of conspecifics) is often mentioned in the popular literature. In the field, this behavior was seldom seen, but evidently occurs more often under aquarium conditions. This is probably due to crowding and lack of sufficient rocks for proper burrow construction. In a 3000 liter laboratory aquarium with a bottom area of nearly one-quarter square meter for each of 15 fish, rock stealing was rare since the aquarium allowed a reasonably natural density of fish.

COMPARATIVE RELATIONSHIPS AND DISCUSSION OF THE ECOLOGY OF *Opistognathus aurifrons*

The sandy areas bordering reefs possess several characteristic species of fishes. Included in this group are the sand tilefish, *Malacanthus plumieri*; the gobies, *Ioglossus calliurus* and *I. helenae*; the bridled goby, *Coryphopterus glaucofraenum*; the garden eel, *Nystactichthys halis*; and *Opistognathus aurifrons*. All are burrowers of one sort or another, and all are colored for concealment against a white sand background. Two species, the gobiid *I. calliurus* and the heterocongrid *N. halis*, are amazingly simi-
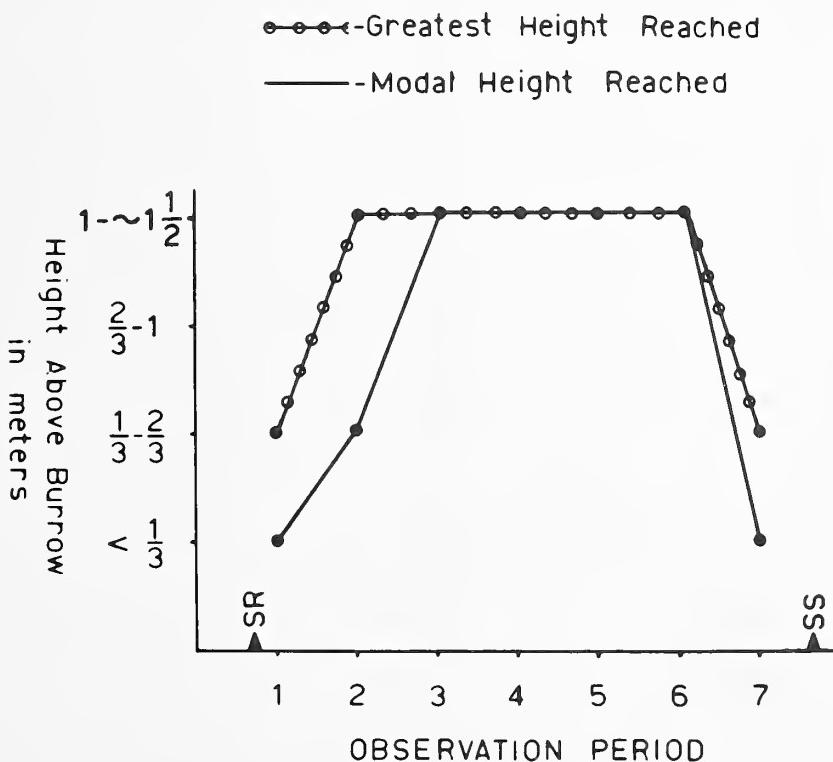
TEXT-FIG. 21. The diurnal patterning of greatest and modal height reached by *Opistognathus aurifrons* above the burrow. Heights above one meter could not be quantitatively measured and were only estimated.

lar to *O. aurifrons* in the way they "make their living."

Specimens of *I. calliurus* known only from Florida waters hover in the water column and probably feed on floating plankton as does *O. aurifrons.* Randall (1967b) reports its West Indian congener, *I. helenae,* to feed entirely on floating plankton. *I. calliurus* enters burrows head-first on the approach of danger and has been seen by the writer performing lateral displays directed toward conspecifics for unknown purposes. Pairs often reside in one burrow which has a narrow vertical tunnel. Often groups of *I. calliurus* were found extremely close to yellowhead jawfish colonies on Florida reefs, but never within their boundaries.

Nystactichthys halis, attaining a length of one-half meter, does not hover as the others, but merely extends a portion of its body out of the burrow (Böhlke, 1957). It picks small zooplankters out of the water column (Randall, 1967a) while remaining partially within its burrows. The position of its eyes, as well as the shape of its pupils, is similar to *O. aurifrons* and probably enables it to utilize binocular vision in picking plankton.

A plankton-feeding existence imposes certain restrictions on the activity of a fish. A major portion of its time must be spent in feeding, due to the small size and spacing of food particles. For example, *O. aurifrons* spends practically 90 percent of the daylight periods feeding. Recent work by Emery (1968b) on the plankton within the reef ledges and caves may modify some of these generalities, since in these localities tremendous amounts of zooplankton are readily available to reef fishes. Most plankton-feeding fishes visually detect their prey. Such feeding is expedited by binocular eyesight and such activity is restricted to diurnal periods. A notable exception to this rule may be the apogonid fishes, which apparently locate plankton visually at night (Randall, 1967a). Most other plankton-feeding fishes are inactive at night.

Some of the western Atlantic congeners of the yellowhead jawfish, *Opistognathus macrognathus, O. maxillosus,* and *O. whitehursti,* differ greatly in behavior, food habits, and coloration. They 1) do not hover; 2) feed primarily on benthic forms and free swimming forms living near the bottom (Randall, 1967a); and 3) are brownish, dusky, or mottled in coloration. In addition they are often found in areas of turbid water.

Nothing is known of the food habits of the congeners of *O. aurifrons* typically found in clear water, i.e., *O. lonchurus* and *O. gilberti.* Both species have been reported not to hover as *O. aurifrons* (*O. gilberti,* Böhlke, 1967; *O. lonchurus,* Böhlke, 1967, W. A. Starck, pers.

comm.), and it seems likely they are not particulate plankton-feeders. *Opistognathus aurifrons* may well be the only plankton-feeding member of the genus *Opistognathus* in the western Atlantic.

The clear-water group of *O. aurifrons, O. lonchurus,* and *O. gilberti* apparently do not overlap in their ecologic distribution. *O. gilberti* is known only from the Bahamas and some areas of the Caribbean, typically on steep slopes in water 28 to 47 m in depth (Böhlke, 1967). *O. lonchurus* may be continental in distribution at depths between 38 and 93 m, and apparently prefers siltier sand conditions than *O. aurifrons* (Starck, pers. comm.). It is not found near the rocky outcrops associated with *O. aurifrons.* The only area along the Florida coastline where such substrate conditions are found in combination with clear water is seaward of the deep reefs, the latter terminating at a depth of about 30 m. It may well be that the distribution of *O. lonchurus* is determined by substrate and water conditions, not by depth. A case in point is seen at Triumph Reef, Florida; substrate conditions similar to those on the shallow reef are found to a depth of 50 m, and in this area *O. aurifrons* is abundant and *O. lonchurus* absent.

Opistognathus macrognathus, a species found in Florida in shallow water, has also been taken occasionally at Triumph and Long Reefs to a depth of 45 m. Several specimens have been taken near to *O. aurifrons* colonies; in one case a specimen was collected from a burrow only 60 cm distant from a yellowhead jawfish burrow.

Few reef fishes live in colonies. One clear exception to this is the yellowhead jawfish. Members of this species are reluctant to stray more than a few meters from their burrow, and this requires that the fish live close together for purposes of reproduction. Competition for food, which might be critical in benthic feeding fishes, is eliminated by the constant influx of plankton with the movement of water immediately above the burrows.

Little is known of the larval life of *O. aurifrons.* Specimens of *O. macrognathus* reared in aquaria metamorphosed from free swimming larvae to burrow dwelling juveniles 18 days after hatching. It seems likely that *O. aurifrons* has a similar larval development. How long individuals can remain as planktonic larvae will, of course, limit the distributional abilities through currents of this species.

Spawning extends at least from spring through late autumn. Fishes brooding eggs have been observed at the following locations on the dates given: Triumph Reef, Florida, May 27; Bimini, Bahamas, May 25, June 2; Serranilla Bank,

Oct. 5. Whether a female will spawn more than once per season is unknown, but it seems likely since multiple spawning of *O. macrognathus* about two weeks apart have occurred in aquaria.

The short larval life of jawfishes is advantageous since this period is the time of greatest predation. Unhatched fish are protected by the brooding parent and the burrowing, mature and immature fish have effective means of avoiding predation. This type of larval life, however, reduces genetic interchange over long distances, and coupled with the reduction in genetic exchange due to the non-wandering habits of the adults may result in the variability of this species over its geographic range. The occurrence of one "Bahama type" specimen in a group of Florida Keys specimens (Böhlke, 1967) may indicate that larvae may rarely get across open water barriers, such as the Florida Current, or that such variants occasionally are produced. Whether this occurs with sufficient regularity to keep the populations from moving farther apart is not known.

The significance of the dark head markings apparently used in behavioral displays and their absence in Florida specimens is not understood. The behavioral actions of Florida specimens are similar, if not identical, with those produced by Bahama specimens. Aquaria, containing members of both populations, might provide interesting insight into differences between these populations, e.g., whether the members of the populations are reproductively isolated for physical or behavioral reasons (presently unknown).

Various morphological adaptations of *O. aurifrons,* not present in the less specialized members of *Opistognathus,* are interesting when viewed from a behavioral-ecologic standpoint. The yellowhead jawfish possesses recurved canine teeth on the lower jaw; yet these are not needed in the capture of food which consists of planktonic animals. It seems likely that these teeth are an aid in carrying large rocks which would otherwise easily slip out of the jaws. This therefore appears to be advantageous for life in a rubble-strewn, calcareous sand area. Similarly in this species, the large mouth is needed not for feeding but appears to be essential only for digging and brooding the young.

The coloration of *O. aurifrons,* unusual among jawfishes, is again an apparent adaptation to the environment. The fish blends well against a white sand background.

Behavior can be thought of as a product of the environment. As previously discussed, the plankton-feeding existence imposes certain requirements on behavior and the burrowing existence also imposes its own set of restrictions. In combination, these restrictions require that the fish have as the spatial center of its activity the burrow, yet spend the major portion of the daylight period in the water column feeding out of contact with the burrow. This results in the retreat behavior observed and accounts for the great wariness of this fish. It also makes colonial existence advantageous with the resultant effects on intraspecific behavior and genetic interchange. An organism reflects the requirements of its environment through appropriate behavior; and this, in turn, is seen most readily through the various adaptations that exist for a given ecological niche. The yellowhead jawfish is a most instructive creature for demonstrating this reflection and the apparent success of its adaptations for its "chosen" niche.

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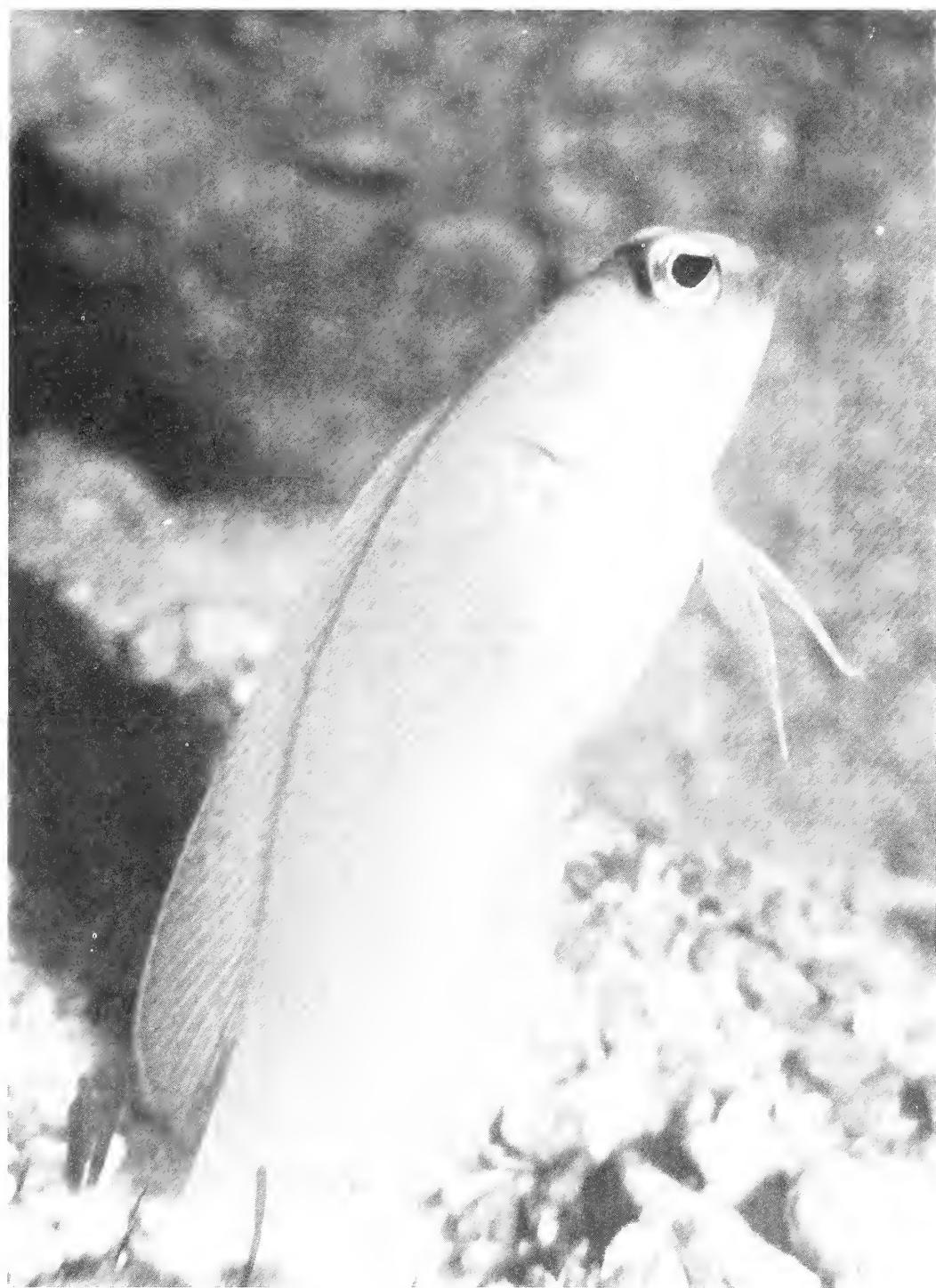
Plate I, Figure 1. A Bahamian specimen of *Opistognathus aurifrons* at the mouth of its burrow.
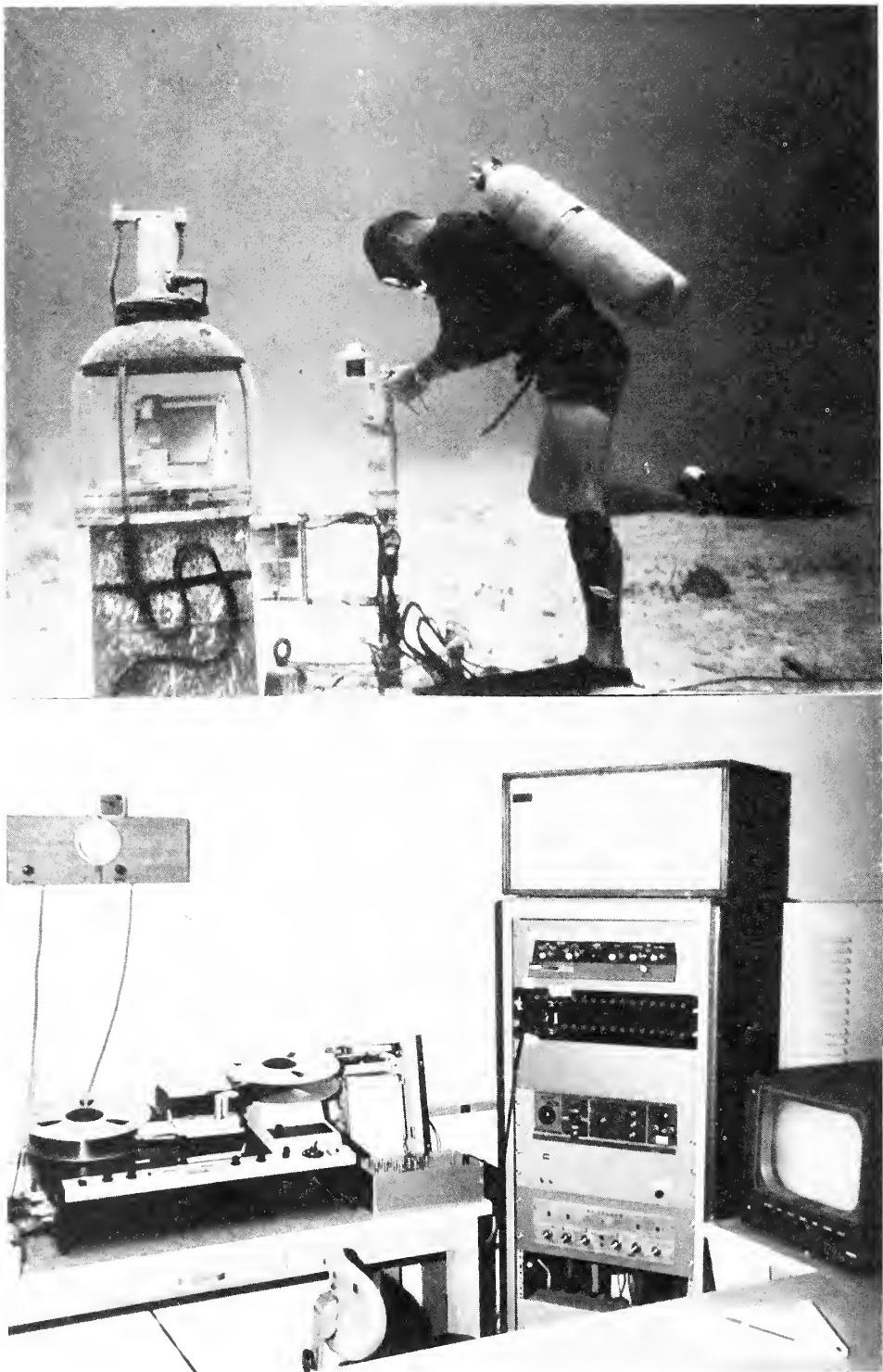
PLATE II, FIGURE 2. Underwater television (UTV) installation located at a depth of 20 m one mile off the coast of North Bimini, Bahamas.

FIGURE 3. Monitor room for the UTV system located at the Lerner Marine Laboratory. Visible are the control console, television monitor, event recorder, and video tape recorder.
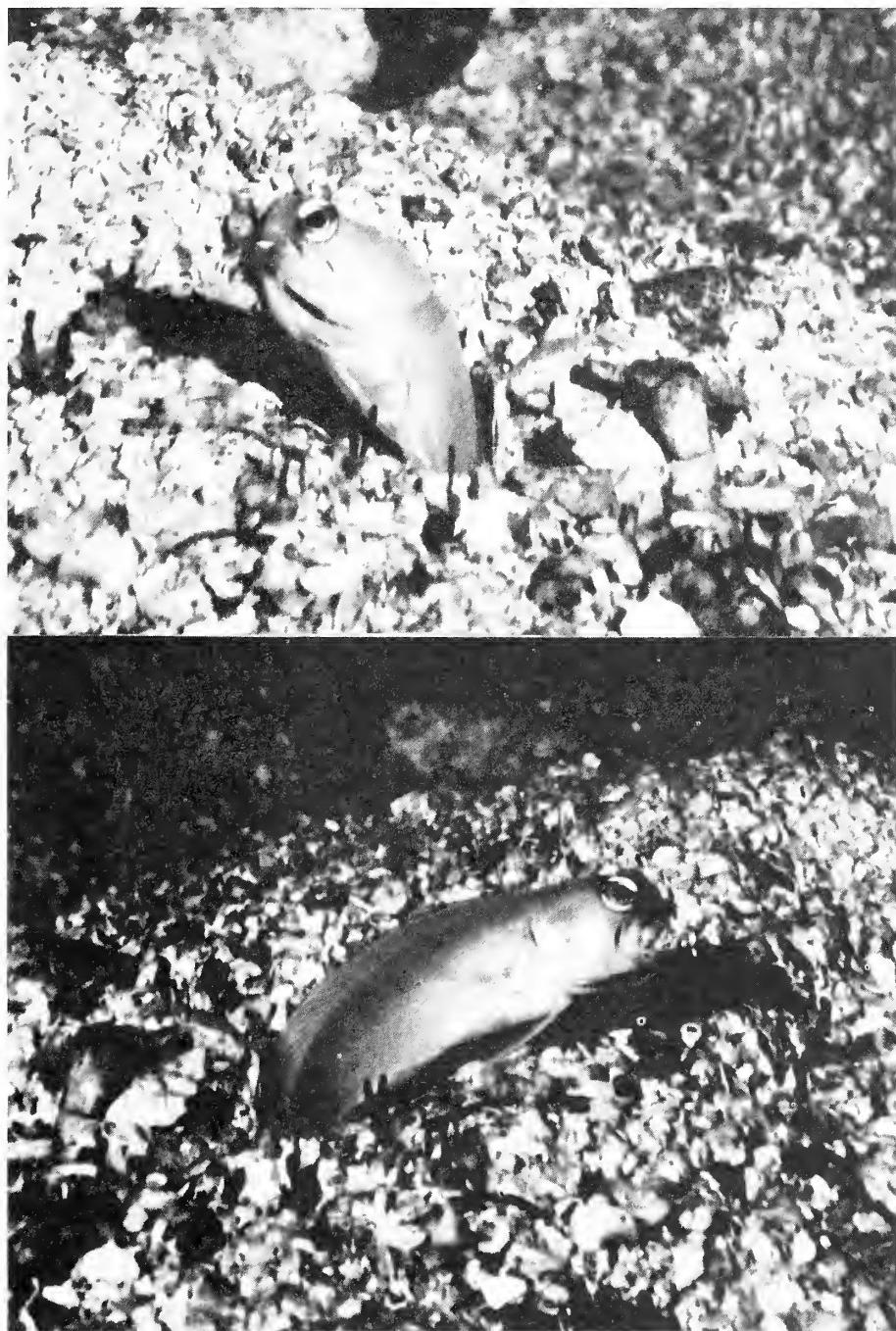
PLATE III, FIGURE 4. The action of "dig" (within burrow) performed by an individual of *O. aurifrons*. The dark line on the isthmus, normally hidden by folds of skin, is clearly exposed.

FIGURE 5. Lateral view of the action of "dig" (within burrow) performed by an individual of *O. aurifrons*. At this point the sand is being expelled from the mouth.

PLATE IV, FIGURE 6. The action of "dig" (retrieve sand) performed by a specimen of *O. aurifrons.* This action is performed some distance from the burrow opening.

FIGURE 7. The action of "retrieve rock" (remove rock from within burrow) performed by an individual of *O. aurifrons.*
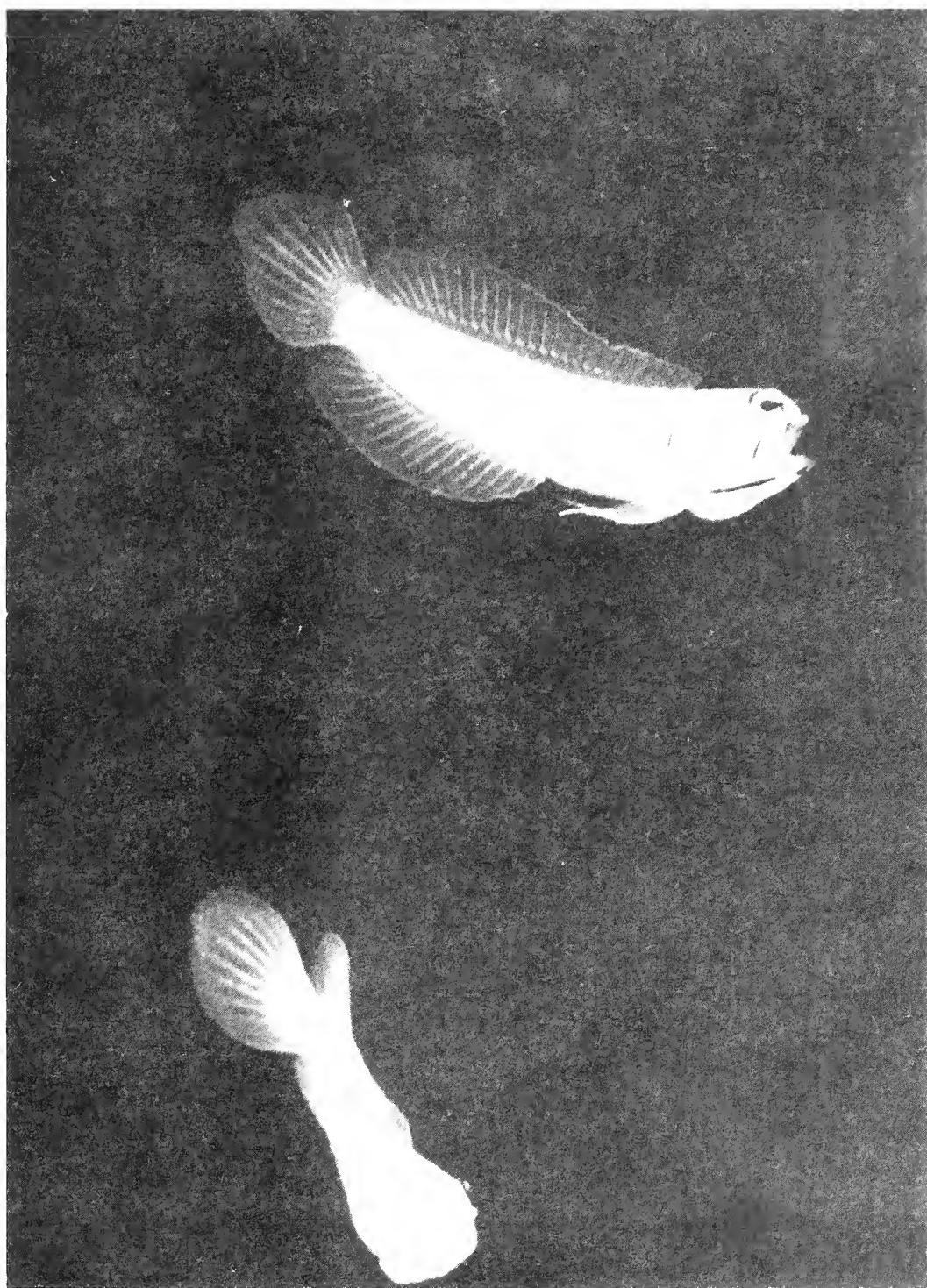
PLATE V, FIGURE 8. The action of "arch" performed by a male specimen of *O. aurifrons*. The male (upper fish) arches the body, spreading the fins, then opens the mouth exposing the various dark lines on the head. The lower fish is a female.

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